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EPINEPHRINE AND THE BLOOD SUGAR LEVEL

CATALDO J. COLETTI, JR.

From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York

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The blood sugar time curve after epinephrine injection in rabbits has been reported in the literature, but none of the work has been carried on with a dose in any way proportional to the body weight. Bang (1) used 1 mgm. per animal, and Cori and Cori (2) 0.5 mgm. per animal. Nor has there been a precise description of the blood changes during the first hour. Further, it does not seem to us that a sufficient number of observations was made to establish a blood sugar time curve.

In the present paper, the blood sugar samples were taken at short enough time intervals to establish more precisely the trend of the blood sugar changes during the first hour after the injection of 0.25 mgm. per kilogram. Also, observations were made at the 30-minute interval, after injection of several other doses.

The rabbits used were selected with regard only to physical condition. The animals were fed on a mixed diet of hay, oats, alfalfa, and molasses. They received carrots twice a week, and water on the other five days. They were confined in individual cages, 12 by 16 by 20 inches, during the period of observation in the laboratory, and while in the animal room.

An inanition period of about 24 hours preceded the epinephrine injection. The blood samples were obtained from the marginal ear vein after hyperemia was produced by the application of a small quantity of xylene.

The total reducing substance of the blood (blood sugar) was determined by Somogyi's (3) micro-modification of the Shaffer-Hartman method (4), using Somogyi's alkaline-copper-iodine reagent no. 1, described by Peters and Van Slyke (5).

The adrenalin used was the product of Parke Davis Co. It was diluted with 0.9 per cent sodium chloride, so that 1 cc. of the resulting mixture contained the dose to be injected per kilogram body weight. The injec-

tions were made subcutaneously in the region of the flank or the abdomen. The injections were followed by a mild massage.

For measuring the precision, the mean deviation $\epsilon = \sqrt{\frac{\Sigma d^2}{N-1}}$, rather than the standard deviation, was used, for the reasons outlined by Scott (6). When the means of the different series are compared, the mean deviation of the mean $\epsilon_M = \frac{\epsilon}{\sqrt{N}}$ was employed.

In the first set of observations, for determination of the time curve, the initial blood sugar was determined before injection of 0.25 mgm. per kilo-

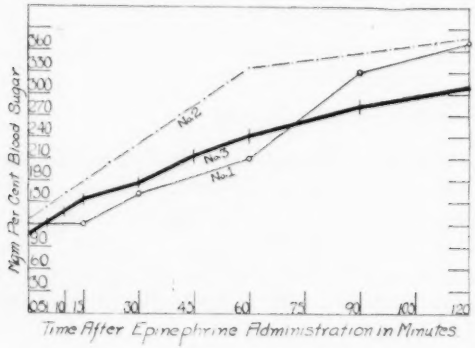

Fig. 1

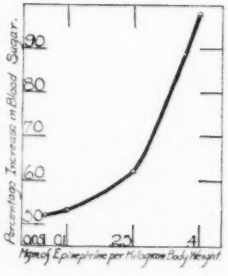

Fig. 2

Fig. 1. Blood sugar time curves for rabbits after subcutaneous injection of epinephrine. Curve 1. Bang. After subcutaneous injection of 1 mgm. per animal. Curve 2. Cori and Cori. After subcutaneous injection of 0.5 mgm. per animal. Curve 3. Results of present investigation, after subcutaneous injection of 0.25 mgm. per kilogram body weight. The vertical lines represent the precision with which the several points were established. The length of the lines represents ϵ_m.

Fig. 2. Percentage change in blood sugar in rabbits ½ hour after subcutaneous injection of various doses of epinephrine.

gram of epinephrine and blood sugar samples were taken at 5, 10, 15, 30, 45, 60, 90 and 120 minutes after injection.

In the second set of observations, the determination of the dose curve, the initial blood sugar was taken, and blood samples taken one-half hour after the subcutaneous injection of 0.05 mgm., 0.1 mgm., 0.25 mgm. and 0.4 mgm. per kilogram body weight.

In figure 1, the change in the blood sugar concentration is plotted against the time after epinephrine injection. Each point is the mean of 50 observations.

The initial blood sugar value of 106 mgm. per 100 cc. of blood compares

with the values varying from 102 to 106 mgm. per 100 cc. for different groups of rabbits, as obtained by Dotti (7).

The 15-minute blood sugar value showed a rise of 46 mgm. per 100 cc. of blood, which does not agree with the work of Bang (1), who showed no change at this time interval, although he apparently used almost twice the dose.

The curve in figure 1 shows a continued rise in the blood sugar up to 2 hours after injection, the rate of change being greatest during the first 15 minutes, and becoming progressively less during the remainder of the time. This is roughly in agreement with the work of Cori and Cori (2) though the time intervals selected by these authors were so great that a smooth curve could not be shown. However it does not agree with the work of Bang (1), who obtained the greatest rate of change in the 60 to 90 minute interval (see fig. 1).

Figure 2 shows the relationship of the percentage change in the blood sugar level to the epinephrine dosage 30 minutes after the administration.

The graph indicates a progressive rise in the percentage change of the blood sugar, as the dose is increased. There was no flattening of the curve with the doses studied. With the doses used, the maximum effect of the epinephrine was not reached. The rate of change was greatest between 0.25 mgm. and 0.4 mgm. This may partially account for the higher blood sugar values obtained by Bang (1) after the 60-minute interval, since he apparently used greater doses.

Apparently from this work the rabbit is more sensitive to epinephrine than is the rat (Hrubetz, 8), for the curve given by the rabbit at this dosage crosses that of the rat after 0.4 mgm. per kilogram, 45 minutes after the injection. Moreover, though the blood sugar has reached a value of 309 mgm. (see fig. 1) per cent at two hours, it apparently has not reached its maximum. The rat on the other hand, after the much larger dose only reached a maximum value of 230 mgm. per cent, and at 2 hours was definitely descending.

Furthermore, in the rat doses over 0.2 mgm. per kilogram failed to raise the blood sugar above the level induced by that dose ½ hour after the injection (Hrubetz, 8). The rabbit, on the other hand, continued to respond to larger doses by an increased blood sugar concentration up to 0.4 mgm. (see fig. 2). Greater doses than this were not studied, though there was no evidence that the limit response had been reached.

The sensitivity of the rabbit to insulin was determined by incidence of convulsion after the injection of 2 units of insulin per kilogram body weight (Dotti, 7).

No relationship was found between the animal's response to insulin and to epinephrine. It may be noted, however, that the females showed a

greater convulsive response to insulin than the males, which is in accordance with the work of Dotti (7).

The author extends generous thanks to Dr. L. B. Dotti for his guidance and advice given throughout this investigation.

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PITUITARY GLAND INFLUENCES ON WATER BALANCE IN THE RAT

H. L. WHITE

From the Department of Physiology, Washington University School of Medicine, Saint Louis

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Since Richter (1) and Pencharz, Hopper and Rynearson (2) have shown that postlobectomized rats always exhibit a permanent polyuria, a natural supposition is that polyuria should appear in hypophysectomized rats on anterior lobe replacement therapy. Pencharz and collaborators were unable, however, to obtain any such effect; the present report presents a confirmation and extension of their work.

1. *Effect of anterior lobe administration on urine output of normal and of diabetes insipidus (postlobectomized) rats.* The postlobectomized rats were operated by Doctor Pencharz about a year before these experiments. The animals were kept in individual collecting cages with water but no food for 21 hours daily. For 6 days at about the middle of the 25 day period each animal received intraperitoneally 0.2 cc. per 100 grams of an acid extract of beef anterior lobe, prepared according to Loeb and Bassett (3), each cubic centimeter representing 0.25 gram of fresh tissue. The average 21 hour urine output of the 3 normals before treatment was 6.0 cc., during treatment 6.6 cc. and after treatment 6.4 cc.; corresponding figures for the 4 postlobectomized were 25, 22 and 26 cc.

2. *Effect of anterior lobe and of anterior lobe plus thyroid on the urine output of hypophysectomized rats.* Four rats, operated about a year previously by Doctor Pencharz, were kept in collecting cages 12 hours daily. In all cases 0.2 to 0.5 cc. of anterior lobe extract per 100 grams for 2 to 4 consecutive days slightly diminished urine output (4.0 to 3.2 cc.). No change was produced by 0.01 gram desiccated thyroid per 100 grams by stomach tube daily for 4 consecutive days. With combined anterior lobe (0.5 cc. per 100 grams) and thyroid for seven consecutive days 1 animal showed a definite rise (4.5 to 10 cc.), the others no change or a slight fall.

3. *Effect of anterior lobe and of thyroid plus anterior lobe on basal metabolism and urine output of normal and hypophysectomized rats.* To test the possibility that an increase in basal metabolic rate may be an essential condition for a diuretic response to anterior lobe administration the urine outputs of 4 normal and 4 hypophysectomized rats were followed in re-

sponse to anterior lobe and to thyroid plus anterior lobe administration, while basal oxygen consumption was followed on 2 of each series. Two of the hypophysectomized rats were operated by Doctor Pencharz, 2 by Dr. Jane Russell; the basal metabolic rates were determined by Dr. R. E. Fisher. Urine collections were made for 15 hours daily. The average 15 hour urine output of the normals before treatment was 4.8 cc., during a 7 day period of 0.5 cc. anterior lobe extract per 100 grams daily 3.5 cc. and during a 5 day period of anterior lobe plus thyroid (0.01 gram per 100 grams) was 3.8 cc. Corresponding figures for the hypophysectomized rats are 6.0, 3.2 and 3.8 cc. In the 2 normals followed, original basal oxygen consumptions were 123 and 132 cc. per 100 grams per hour, were raised by anterior lobe administration to 157 and 153 cc., and on administration of anterior lobe plus thyroid were 142 and 153 cc. Corresponding figures for the 2 hypophysectomized followed were 104 and 88, 104 and 140, 140 and 182 cc. It is thus seen that a failure in the rat of diuretic response to anterior lobe and to anterior lobe plus thyroid cannot be ascribed to a failure of increase in basal metabolic rate.

In no case did anterior lobe administration increase urine output of normal, of postlobectomized or of hypophysectomized rats; there was rather a decrease. Thyroid administration did not increase the urine output of normal or of hypophysectomized rats. Thyroid plus anterior lobe administration was without significant effect on urine output of normal rats; in 1 case of 7 in hypophysectomized rats it was accompanied by a significant increase in urine output, in the other 6 by a fall of variable extent. It is obvious that the rat is much less sensitive than the dog to the diuretic action of beef anterior lobe extract; it rather resembles the monkey (4).

SUMMARY

The rat does not respond as does the dog with an increased urine output to administration of beef anterior lobe extract or of anterior lobe plus thyroid. The usual response of urine output of normal, of postlobectomized and of hypophysectomized rats to administration of anterior lobe extract alone or with thyroid is no change or a decrease. Failure of increase in urine output cannot be ascribed to a failure of increase in basal metabolic rate.

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VARIATIONS IN ALVEOLAR CARBON DIOXIDE IN MAN DURING HUNGER

ROLLAND J. MAIN

From the Department of Physiology, Medical College of Virginia, Richmond, and the University of Chicago

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Although the internal environment of the human body is kept constant within remarkably narrow limits, yet this constancy is not necessarily maintained by any immutability of the individual factors concerned, but is rather due to the fact that their resultant tends to remain unchanged. Therefore, we may expect that the term "physiologic constant," so often applied to these single factors, may merely reflect upon the technic or sensitivity of the apparatus used for their determination. Thus, the tension of CO_2 in the alveolar air of a man awake is generally stated to be constant. However, Porges (1911), Erdt (1915), Essen et al. (1921), Dodds et al. (1921a, b, c; 1923), Endres (1922), Veil (1922), Jansen and Karbaum (1926), and Lewin (1926) claimed that this is only true of a fasting human subject until he partakes of food. Then, because of the secretion of gastric juice and the consequent loss of hydrogen ions from the blood, an alkalemia is produced and the carbon dioxide of blood and alveolar air rises. Dodds (1921a, b) also claimed that because of later pancreatic secretion and the resulting loss of alkali, an acidemia then occurs, and the carbon dioxide content of alveolar air falls. Following the reabsorption of the alkaline juices, equilibrium is again restored, and the alveolar carbon dioxide maintains a level until the subject eats again.

I attempted to utilize these supposed changes in alveolar carbon dioxide as an index of gastric and pancreatic secretion in man, and first began the determination of the expected level of alveolar carbon dioxide in the fasting patient. However, I found that tremendous changes in the carbon dioxide content occur when fasting, especially during the time at which a meal would ordinarily be ingested. These results were so at variance with those of Dodds especially, that my original purpose was discarded in order to investigate the cause of these variations, especially since Dodds' conclusions had already been criticized by Brunton and Israels (1930), as were those of Erdt (1915) by Van Slyke et al. (1917).

METHOD. A modification of the Haldane-Priestley (1905) method was utilized in collecting samples of alveolar air. A metal mouthpiece was

attached to a rubber tube 2.5 cm. in diameter and 1.2 meter in length. At right angles to the side of the mouthpiece was a short side tube to which an evacuated 80 cc. gas tube could be affixed, with a minimal dead air space. The patient was instructed to respire through his nose until he was sure he was breathing at a normal rate and depth. This is a necessary precaution to prevent nervousness from affecting his respiration. When he felt that his respiration was quite normal, at the end of a normal expiration, he forcibly, rapidly, and completely, emptied his lungs, forcing out his reserve air into the tube. He then placed his tongue against the slotted mouthpiece to prevent leakage, and raised his hand. The experimenter then turned the cock of the evacuated gas tube attached to the mouthpiece, and the last 80 cc. of air expired were removed for analysis. This procedure gives alveolar air samples which agree with the gas tension of arterial blood much better than either inspiratory samples or averages of both inspiratory and expiratory samples (Bock and Field, 1924). All analyses were made on the large model Haldane gas analyser, using approximately 20 cc. of gas.

This procedure for the collection of alveolar air is surprisingly accurate. Often, in order to check the accuracy of collection and determination, three consecutive samples were collected as rapidly as possible, and each sample was analyzed twice. The CO_2 content of the three samples usually checked within less than 0.1 per cent. However, it is necessary to employ intelligent subjects, and some previous experience in collecting the sample is preferable. Having found the average ward patient to be unsuitable for these reasons, I have restricted this investigation to my colleagues and students.

All determinations were made on the subject lying comfortably in bed or reclining in an easy chair and cautioned against moving except as necessary. This was done in order to obviate any possible changes in blood or alveolar CO_2 due to excess production of CO_2 or acid by muscular activity, and to possible proprioceptive stimulation of respiration. Smoking was not permitted. The bladder was emptied every hour or two, and the subjects were warned against becoming drowsy. On a fasting patient it is preferable to make determinations every 10 or 15 minutes, because of the rapid changes that occur at times. In fact, it would often be of advantage to make analyses 5 minutes apart, but this is practically impossible unless two operators and two sets of apparatus are available.

When gastric contractions were recorded in some of the experiments, a small rubber balloon was tied to an Einhorn duodenal tube and swallowed. A water manometer was used for recording contractions.

Results. The alveolar CO_2 curves of five fasting subjects are shown in figure 1. Great variations are apparent among the curves of different subjects, but it can easily be seen that a distinct drop usually occurs at

meal times, followed by a rise. The extent of these drops varies among the subjects, but is fairly constant in each one.

These peculiar variations of CO_2 seemed inexplicable after the possibility

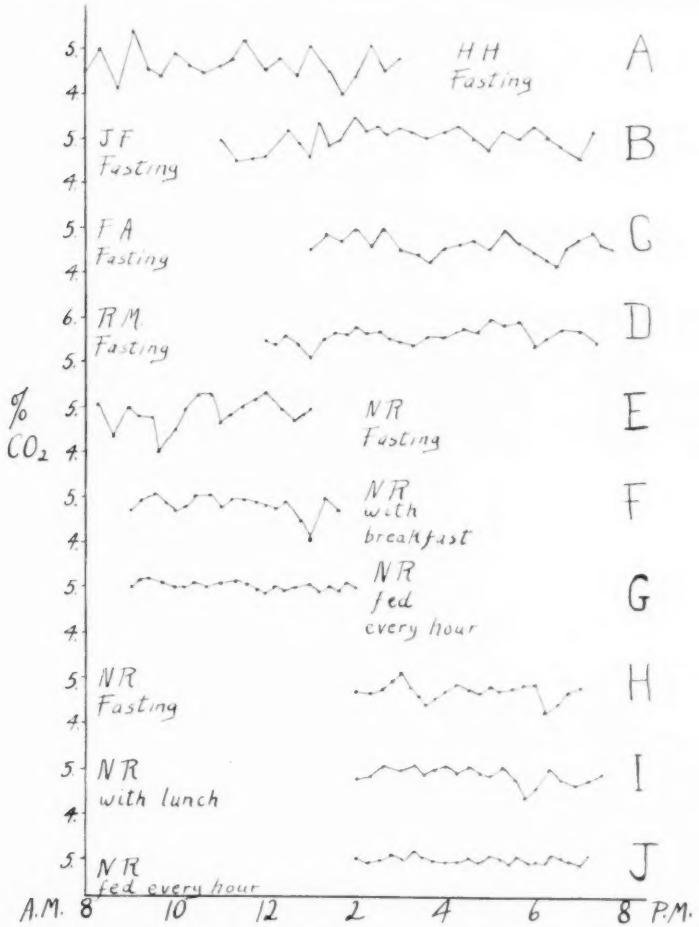

Fig. 1. The variations in alveolar CO_2 in fasting subjects as compared to fed subjects.

of experimental errors was ruled out. However, because of the tendency of the curve to fall most about the time during which a meal is ordinarily ingested, it was thought that perhaps hunger pangs might be responsible. A possible appetite secretion of gastric juice could not be blamed, for this

would have caused an alkalosis and a *rise* in alveolar CO_2, rather than the observed drop. Moreover, it would seem impossible for such large quantities of acid to be secreted by a fasting subject so rapidly as to produce these marked changes in the CO_2 equilibrium of the blood. Besides this, the curves of two subjects with achlorhydria showed similar variations.

Carlson (1916) and Hoelzel and Kleitman (1927) stated that gastric hunger contractions will markedly stimulate respiration, and Nechoroschew (1929a, b) observed a disturbance of respiration during hunger contractions in cats and dogs. That a correlation between gastric hunger contractions and respiration exists, was verified in several subjects by recording gastric motility and respiratory movements simultaneously.

The effect of gastric hunger contractions on respiration is quite variable. Sometimes a relative apnea occurs during the contraction, to be followed

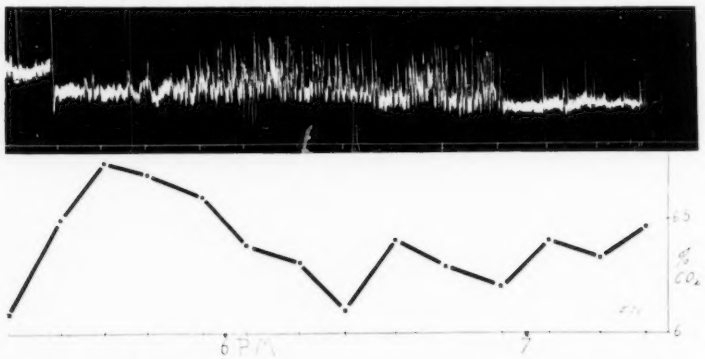

Fig. 2. Correlation of gastric hunger contractions (upper graph) and alveolar CO_2 (lower graph) (in per cent of an atmosphere pressure). At 6:35 p.m. the rise in CO_2 is accompanied by a temporary diminution of contractions.

by polypnea and hyperpnea. Sometimes hyperpnea alone occurs during the contraction, so that the maximal respiratory effort coincides with maximal gastric contraction.

However, the relationship between respiration and hunger does not necessarily imply a *stimulation* of respiration, but merely a *disturbance*. It was necessary, then, to plot hunger contractions and the alveolar CO_2 simultaneously, as was done in several subjects, with the usual result as shown in figure 2, which indicates that coexistent with a period of hunger contractions, there must exist a true stimulation of respiration, since alveolar CO_2 is lowered.

If hunger contractions are then correlated with a stimulation of respiration, the abolition of those contractions should result in a more constant alveolar CO_2 level. In order to inhibit hunger, I allowed subjects to eat their regular meals (F and I, fig. 1) and to ingest a glass of milk and two

Uneeda crackers every hour (G and J, fig. 1). The constancy of the curve always obtained by this means supports the idea that hunger is probably responsible for the drops in the fasting subject.

Discussion. Since during hunger contractions, there exists a disturbance of respiration accompanied by a drop in alveolar CO_2 tension, we may postulate that hunger contractions actually stimulate respiration, perhaps through activation of sensory end organs in the stomach wall, which either directly stimulate the respiratory center, or merely increase its sensitivity to CO_2. This hypothesis is supported by the fact that each individual hunger contraction is accompanied by a simultaneous effect upon respiration, as if a nervous mechanism were at work, i.e., a visceral control of respiration. Essen et al. (1921) also noticed marked variations in alveolar CO_2 during fasting, which disappeared following pyloric resection. This would tend to substantiate the idea that hunger contractions are directly responsible for respiratory stimulation, if we consider that pyloric spasm produces more stimulation than contraction of the fundus. Morin and Vial (1934) also proved the existence of a visceral effect upon respiration by distention of the intestines of dogs.

On the other hand, it is possible that some humoral factor responsible for production of hunger contractions, as hypoglycemia or a "hunger hormone," or a coincident state, as a fasting ketosis, may likewise stimulate respiration. This idea is supported by the fact that there is not always a perfect correlation between the drop in CO_2 and the duration of hunger contractions. Sometimes the CO_2 does not rise immediately upon abrupt cessation of gastric peristalsis.

Since there is a lowering of alveolar CO_2 during hunger, we could expect either 1, a resultant alkalemia due to CO_2 removal, or 2, a primary acidemia which has caused CO_2 excretion. Attempts to analyze this point by determining the pH of the blood by the indicator method have not shown a shift in either direction, and have been complicated by the fact that venepuncture usually inhibits gastric contractions. However, Shock and Hastings (1934, figs. 7 and 8) in a study of diurnal changes in blood, found that in some subjects there is marked rise in blood pH prior to the noon meal, accompanied by a fall in CO_2 tension.

Previous claims that alveolar CO_2 rises after a meal may well be due to the fact that prior to the meal CO_2 is low due to existing hunger, and is high afterwards merely because of the inhibition of hunger contractions, with a diminished stimulation of respiration. One must avoid, however, the tendency of the subject to become drowsy following eating, since alveolar CO_2 rises during sleep (Straub, 1915). In view of the data obtained in this investigation, the rise in alveolar CO_2 after a meal cannot be ascribed solely to the secretion of HCl: a conclusion which appears to be accepted at present (Peters and Van Slyke, 1931). It may well be that gastric secretion does affect the tension of CO_2, but since previous workers

were unaware of the disturbing effect of hunger contractions, the question remains open.

The minor variations in CO_2 tension still occurring after hunger is abolished by feeding (G and J, fig. 1), may be due to variations in the sensitivity of the respiratory center, or to rhythmical stimulation by other hollow viscera.

The marked variations in respiration and alveolar CO_2 of a fasting subject indicate that the basal metabolic condition is not always basal for respiration, and that alveolar CO_2 is not a physiological constant, but is rather the function of all factors acting upon the respiratory center.

SUMMARY

Marked variations occur in the alveolar CO_2 of fasting subjects, especially at the usual meal hour. The evidence indicates that these changes may be due to a respiratory stimulation concomitant with hunger contractions, rather than being dependent upon secretion of digestive juices.

I am deeply indebted to Drs. A. J. Carlson, A. B. Luckhardt and N. Kleitman for their generous material aid and advice, and to Miss M. K. Cary for technical assistance.

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INCREASED ACOUSTIC SENSITIVITY IN DOGS FOLLOWING ROENTGEN-RADIATION OF THE HYPOPHYSIS[1]

W. J. BROGDEN[2] AND ELMER CULLER

From the Johns Hopkins University School of Medicine, and the University of Illinois[3]

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A number of clinicians, notably Richardson (20), Kahn (14), Stokes and Stokes (21), McCoy (17), Pacini (18), Warurck (22), Kinney (16) and Jarvis (13), have reported that partial deafness could be ameliorated by small doses of Roentgen-radiation, when the x-ray beam was directed anywhere upon the head. Not all patients showed the effect, however, and in many cases the improvement was but temporary. The only experimental study is due to Girden (9) who, working in this laboratory, investigated the problem with dogs whose auditory acuity could be accurately tested by the conditioned-response technique. He reported a transient gain of about 5.5 decibels at 1000 cycles following exposure to small doses of Roentgen-rays. The increased sensitivity appeared in 8 dogs after a latent period of some days and continued for 2 weeks more or less; thereafter the animals' limens reverted to normal.

The present investigation has had several objectives. 1. The basic phenomenon (increase of acoustic sensitivity after Roentgenization) was critically reëxamined. 2. Three test frequencies (125, 1000 and 8000 cycles) were employed instead of one. 3. A possible correlation between dosage of Roentgen-radiation and magnitude of hearing-gain was examined. 4. The principal objective, finally, was to locate the site or source of the effect, whether systemic or local, and if local, whether central or peripheral; and to trace the mechanism whereby it comes to pass.

APPARATUS AND TESTING PROCEDURE. The animal's acoustic limen was

[1] Communication no. 28 from the Physiological Psychology Laboratory, University of Illinois, established and maintained with aid from the Research Council, American Otological Society. Grateful acknowledgment is made to the Josiah Macy, Jr., Foundation, whose financial aid made this research possible.

[2] National Research Council Fellow in Psychology, Pavlovian Laboratory of the Phipps Psychiatric Clinic, The Johns Hopkins Hospital.

[3] With the collaboration of Prof. G. L. Clark, Director of the x-ray laboratories of the Department of Chemistry, who offered the use of his excellent facilities and contributed many helpful ideas about procedure and interpretation. Dr. W. S. Coe, one-time member of his staff, aided considerably in the technical phases of the problem.

always determined by a form of the motor-conditioning method (described in ref. 7); which is fully equal in precision to the standard psychophysical techniques with competent human subjects. A deep-therapy Coolidge tube with tungsten-target was used throughout for Roentgen-exposure, the tube being operated always at 90 kv. and 5 milliamperes. The intensity of the x-ray beam is here expressed in Roentgen-units,[4] measured by means of a Victoreen r-meter. Dosage in every case is a linear function of duration of exposure. The animal's head was secured in a holder, 30 cm. from the target; general anesthesia (Nembutal) was employed during the long periods (6 to 13 min.) needed for heavy doses. From the lambdoidal ridge forward the head was exposed to the x-ray beam through a rectangular aperture of 100 sq. cm. in the heavy lead-sheet which protected the rest of the body. The control animals received identical treatment in every

TABLE 1

	HEARING-THRESHOLDS			BLOOD-SUGAR
	125 cycles	*1000 cycles*	*8000 cycles*	
1. Pre-radiation				
Mean	77.2	104.0	92.0 db	82.10 mgm./100 cc.
Standard error	0.40	0.63	1.00	1.38
2. Post-radiation				
Mean	84.4	112.8	100.0	78.05 mgm.
Standard error	0.75	0.48	0.63	0.46
3. Differences				
Mean	7.2	8.8	9.6	−4.05
S.E.	0.85	0.79	1.18	1.45
t (= Mean/S.E.)	8.47	11.14	8.14	2.79
p	<0.001	<0.001	<0.001	0.012

detail, including anesthesia, except that the head also was fully protected with lead.

In order to determine the statistical "significance" of the change in hearing following Roentgen-exposure, the limens were divided into groups of five, counting backward and forward from the first day of radiation. The average pre-radiation thresholds were then compared with the post-radiation means by computing both the differences between them and the standard errors of these differences. The ratio of any difference to its

[4] "One roentgen, or r, is obtained from that x-ray energy which, when the secondary electrons are fully utilized and secondary radiation from the wall of the chamber is avoided, under standard conditions 0°C. and 760 mm. of mercury pressure, produces in 1 cc. of atmospheric air such a degree of conductivity that the quantity of electricity measured at saturation current equals one electrostatic unit" (4, p. 163).

own standard error is here called t. With the t value, one can enter "Student's Tables" (8) and find the p value. When p is less than 0.02 (which signifies that the difference in question might be expected twice in 100 samples drawn at random from a homogeneous population) the difference is regarded as "significant." A complete description of this statistical approach is given by Fisher (8).

Discussion of results. Our first objective was to explore two questions evoked by Girden's study. *a.* Is the apparent gain in hearing a true *sensory* effect, or is it due merely to heightened motor responsiveness of the animal following radiation? On this question we have two convergent lines of evidence. 1. Does Roentgen-radiation, as a matter of fact, induce restlessness in the animal? By use of a polygraph, with appropriate relays and switches, the complete test-situation (tonal stimulus, shock, every

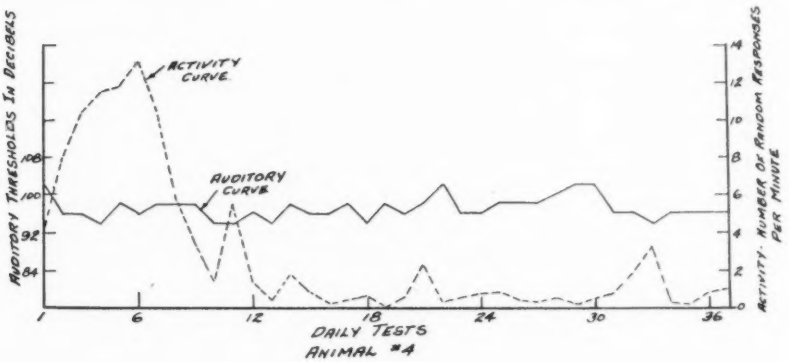

Fig. 1

movement of the conditioned limb) was projected upon a moving-tape record. Full records were thus obtained for seven dogs at three frequencies: twelve from four animals which were, and nine from three dogs which were not, radiated over the brain. Of the first twelve records, six were positive (increased activity after radiation), six were negative; of the second nine, four were positive, five negative. It appears that there is no change in spontaneous activity of the conditioned limb subsequent to radiation; x-rayed animals behave in this regard precisely as do controls. 2. Even if random activity were increased by Roentgen-radiation, would the animal's apparent acoustic threshold, as measured by our methods, be affected? Figure 1 displays the polygraph-record of dog 4 at an earlier time, together with its acoustic thresholds during the same period. The extreme change in random activity, be it noted, was *not* due to x-rays. Whereas the activity-record fluctuates widely, the hearing-curve remains

horizontal throughout. From 1 and 2 we thus have double assurance for the *sensory* character of the x-ray effect.

b. Several roentgenologists expressed doubt that the dosages employed in Girden's study (less than 100 roentgen-units in most cases) or in this study (75 to 675 r-units), generated under the conditions above stated, are of sufficient potency and magnitude to traverse and affect the cranial contents of a dog. A good x-ray photograph of a live dog's head, taken under the regular conditions of radiation, was obtained with an exposure of only one-half second (49/120 of 1 r-unit); a film exposed for 5 seconds was darkened into a uniform dead black. It follows that the dosages here

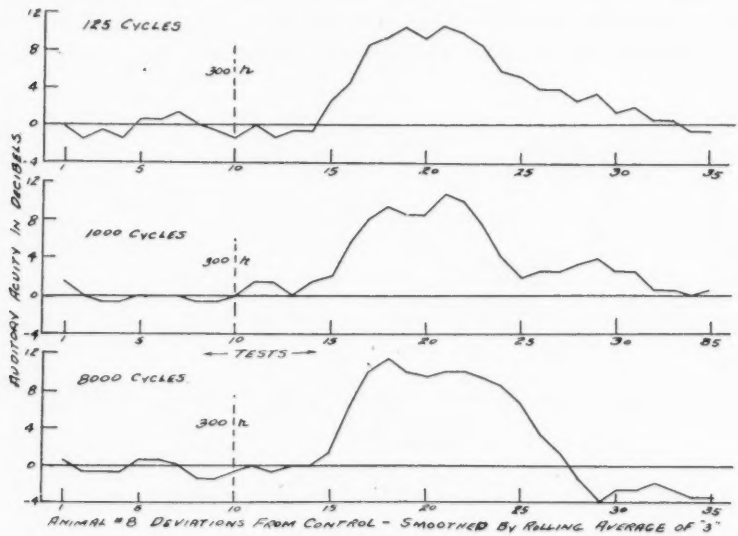

Fig. 2

employed are more than ample to penetrate the entire cranial cavity of a living dog.

The second and third objectives will now be considered. Ten animals were radiated at nine different intensities, ranging from 75 r to 675 r, in steps of 75 r, to discover whether there is any relationship between the magnitude of hearing-rise and the quantity of Roentgen-exposure. Results are briefly as follows. The first figure in each pair gives dosage in r-units, the second gives the average hearing-gain after radiation (mean subsidence of the threshold in decibels for the test-frequencies 125, 1000 and 8000 ~). With 75r, 5.80 db; with 150 r, 4.78; with 225 r, 5.64; with 300 r, 7.53; with 375 r, 7.87; with 450 r, 3.84; with 525 r, 5.69; with 600 r,

7.42; with 675 r, 4.78. The control-dogs, on the contrary, receiving identical treatment in every detail save radiation, changed during the same period −0.66 db. The x-ray effect was not quite equal at all frequencies, being (mean for all test-animals) 5.42 db at 125, 5.88 db at 1000, 6.00 db at 8000. These figures demonstrate *a*, that size of dose has no relation to magnitude of hearing-gain. The greatest change appeared with 375 r and the least with 450 r; the minimal dose depressed the threshold by 5.80, the maximal dose by only 4.78 db. The Pearsonian correlation-coefficient is wholly negligible, being 0.01 with standard error 0.33. The change in threshold is therefore all-or-none in character, its magnitude being fairly constant whatever the dosage (within the range here employed) Figure 2 is offered as a true picture of acoustic sensitivity before and after x-radiation. *b*. The effect is much the same at low, intermediate and high frequencies. Though somewhat less at 125 ~ than thereabove, the difference is statistically undependable.

Being a temporary phenomenon, the duration of this effect requires brief notice. The first figure of each pair gives dosage in r-units, the second figure gives mean *duration in days* of the post-radiation effect: with 75 r, 10.0 days; with 150 r, 10.2; with 225 r, 10.3; with 300 r, 9.0; with 375 r, 9.6; with 450 r, 8.0; with 525 r, 9.6; with 600 r, 9.3; with 675 r, 8.6. At 125 ~ the mean duration is 8.7 days, at 1000, 9.9 and at 8000, 10.0. It thus appears *a*, that duration of the acoustic effect has no appreciable relation to magnitude of dose. The Pearson-coefficient is again wholly negligible, being −0.04 with s.e. 0.33. This confirms the all-or-none character of the phenomenon. *b*, 125 ~ is here also inferior to the higher frequencies; in this case the difference, being over twice its own standard error, is possibly but not surely acceptable.

In each case a latent-period intervened between dosage and inception of the acoustic effect. The first figure of each pair gives dosage in r-units, the second gives mean *latency in days*: with 75 r, 7.6 days; with 150 r, 3.5; with 225, 7.6; with 300 r, 5.6; with 375 r, 5.0; with 450 r, 3.0; with 525 r, 2.3; with 600 r, 2.3; with 675 r, 2.6. At 125 ~ mean latency was 4.5 days, at 1000, 4.5 days and at 8000, 4.0 days. From this we see *a*, that latency of the phenomenon, in marked contrast with magnitude and duration, is sharply contracted as the quantity of radiation rises. The Pearson-coefficient is −0.79 with s.e. 0.13; being six times its own s.e. the correlation may be unhesitatingly accepted as real. *b*. There is here no distinction between frequencies, all having about the same delay.

Having briefly analyzed several characteristics of the phenomenon, we proceed to our fourth objective. As a means of locating the site of this effect, two animals were radiated, with 150 r-units each under standard conditions, one over the hindquarters and one over the thorax. The mean hearing-change in the first dog was 0.57 db, in the second was −0.27 db.

The controls tested concurrently changed by −0.44 and −0.03 db. None of these figures differ statistically from zero. It thus develops that the effect is not general or systemic but appears only upon Roentgenization of the head.

Upon examining the head-region for mechanisms which might be responsible, the pituitary gland appeared most likely for the following reasons. 1. Whereas nervous and brain tissue are highly resistant to x-ray effects (3, 19), the endocrine glands are among the most susceptible of all tissues. 2. In the clinical articles we frequently read that the beam was focused on sella turcica, seat of the gland. 3. Several investigators (Stokes and Stokes, Warurek) suggested that this effect might be due to the hypophysis, without presenting any evidence to substantiate their conjecture, which was not accepted by Carlson (2).

In order to test the pituitary hypothesis, two animals, 17 and 18, were hypophysectomized by the temporal approach.[5] After operation they were conditioned and their auditory thresholds measured daily for a period of some weeks. They then received 150 r-units upon the head under standard conditions. The mean hearing-change after radiation proved to be −0.74 and −0.43 db respectively; the control changed by −0.10 db. No change at any frequency was statistically valid. Histological[6] examination of animal 17 revealed a small piece of hypophyseal tissue about 1 mm. in diameter, resting in the sella turcica. It had been separated from its connection with the hypothalamus, its cells had undergone histolysis and the cell-walls were broken down. Similar examination of animal 18 revealed a short stump of the infundibulum remaining. Serial sections of the region between posterior margin of the optic chiasma and anterior margin of the mammillary bodies disclosed a short length of the infundibulum undetached from the hypothalamus, with no glandular tissue in evidence. The distal end of the lumen was closed over. Hypophysectomy was thus, in both cases, shown to be complete.

It follows that Roentgen-rays act in some way upon the pituitary body to bring about the transitory gain in acoustic sensitivity. Radiation of hypophysectomized dogs reveals no trace of this effect.

If the pituitary body is the crucial factor, how does it operate to improve hearing? It is well known that hypophysectomy makes the dog hyperinsulinic (1, 5, 6); the animal will enter hypoglycemic shock if deprived of food for twelve hours or more. Dr. F. L. Ruch informed us that diabetic patients in the ward of a certain hospital had observed that they could not hear the ticking of a clock on the wall when it was time for them to

[5] The authors are indebted to Dr. B. O. Barnes of the University of Chicago for his aid and suggestions in developing the operative technique for hypophysectomy.

[6] The authors wish to express deep appreciation to Mr. Harlow Ades for making the histological examinations.

take their insulin (high blood sugar), but that when the insulin took effect, the clock could again be heard (lowered blood sugar). Hart (12) reported the case of a diabetic patient who showed a loss of 31 per cent for the right ear and 21 per cent for the left ear by the audiometer. After treatment for diabetes, the patient was again tested two months later and showed an improvement of 13 per cent in the right ear and 8 per cent in the left. Gottlieb (10) reported that in a number of cases of pancreatic insufficiency and hepatic dysfunction, where the blood sugar was raised above normal owing to disturbance of carbohydrate metabolism, the patients showed marked hearing losses. He stated that foods of high carbohydrate content, such as chocolate and ice cream, frequently aggravated these conditions. With treatment (feeding desiccated whole pancreas by stomach tube) both the metabolic derangement and the hearing were improved.

The two hypophysectomized dogs also exhibited an improvement in hearing, about forty days after removal of the gland, comparable in magnitude to the effect shown by normal dogs shortly after Roentgenization. At 1000 and 8000 cycles their thresholds subsided about 6 to 8 decibels; at 125 ~ the decline equaled 4 or 5 db. in one dog and was negligible in the other. It will be recalled that the x-ray effect was likewise a bit less at 125 ~ than thereabove. The reader will note that this change is not an example, but rather a continuance, of the x-ray effect proper. Their relation, and the common mechanism by which they arise, appears to be as follows. Hypophysectomy frees the pancreas from pituitary-inhibition, with resultant over-secretion of insulin. A mild hypoglycemia ensues, the excess of insulin being partially but not wholly compensated by constant withdrawal of glycogen from liver and muscles. After Roentgenization likewise a mild hypoglycemia (about 4 mgm. below normal sugar; cf. table 1) occurs; spreading throughout cerebrospinal and cochlear fluids, this low sugar-level reduces the acoustic threshold. This is the true "x-ray effect." The present dogs being untrained prior to hypophysectomy, their pre-operative thresholds are not available for comparison; but presumably their hearing within the first days after operation was better than normal, owing to the limited hypoglycemia, just as after Roentgenization. Their limens were in fact about 5 decibels below those of the control dogs. But meanwhile the glycogen-supply is being constantly depleted without replenishment; in due time (40 days) the animal's reserves are so exhausted[7] that it must depend upon food-intake alone

[7] When these dogs were dispatched, samples of the liver were examined for glycogen. Determinations by a modified form of Pflüger's method gave 0.68 per cent for no. 17 and 1.81 per cent for no. 18. Inasmuch as the glycogen-content of liver from intact laboratory dogs ranges from 3 to 12 per cent (11), these hypophysectomized animals were obviously far below normal. Corkhill, Marks and White (5) have also shown that glycogen-content of liver of hypophysectomized rabbits is markedly lower than normal.

for maintaining its sugar. Thereupon a more severe and chronic depression of sugar-levels occurs throughout blood, cerebrospinal system and cochlear lymph; and the acoustic threshold subsides still further to a new low level ("hypophysectomy effect").

To examine more fully the above inferences, hearing-tests were run upon both a diabetic subject and a normal dog (no. 20) when blood-sugar was high and when it was low. Blood-sugar determinations were made for the diabetic by the Folin-Wu macro-method and for the dog by the Folin-Malmros micro-method on samples withdrawn during the hearing-test each day.[8] With both subjects, low sugar-levels were produced by intravenous injection of insulin. High blood-sugar in the diabetic was induced by omitting the dose of insulin at the morning meal which contained circa 95 grams of carbohydrate; with the dog, glucose was introduced by stomach tube directly into the gastro-intestinal system. By sampling the blood at 15-minute intervals after glucose ingestion, we learned by preliminary trials that blood-sugar rose to a peak within some 90 minutes and then began to recede; after injection of insulin intra venam, blood sugar reached its minimum within 15 to 30 minutes. Hearing-tests were so timed as always to coincide with maximal hyper- or hypo-glycemia. At the time we failed, however, to allow for the additional time-lag in the cochlear lymph, which is critical for the hearing-effect; for this reason the following data fail to reflect the full force of the sugar-change. Results for the dog are briefly as follows. The normal thresholds (blood-sugar circa 82 mgm.) were 77.5 db at 125 ˜, 101.0 db at 1000 ˜, 80.0 db at 8000 ˜. With high blood-sugar (mean value of 157.7 mgm.) the respective figures were 76.5 db, 98.5 db and 78.5 db; with low blood-sugar (mean value of 66.3 mgm.) the figures were 81.5 db, 109.0 db and 85.0 db. The differences between high and low sugar are, therefore, 5.0 db (s.e. = 2.27) at 125 ˜, 10.5 db (s.e. = 0.81) at 1000 ˜, 6.5 db (s.e. = 1.07) at 8000 ˜. For the diabetic the figures are: with high blood-sugar (mean value of 289.6 mgm.), he tested 78.0 db at 125, 102.8 db at 1000, 82.0 db at 8000; with low blood-sugar (mean value of 69.1 mgm.) the figures are 79.6, 106.0 and 84.0 db. The differences are 1.6 db (s.e. = 2.47) at 125, 3.2 db (s.e. = 1.19) at 1000, 2.0 db (s.e. = 1.78) at 8000. From these figures we see *a*, that change in sugar-level produces at each frequency a change of the same sign in threshold of hearing. High sugar raises the limen above normal, low sugar abates it; the effect being more marked however in the latter case. With this dog the thresholds during low sugar are less than normal by 4.0 db at 125 ˜, by 8.0 db at 1000 ˜, and by 5.0 db at 8000 ˜, a mean reduction of 5.67 db; after Roentgenization (cf. p. 17 above) the corresponding figures are 5.42 db, 5.88 db and 6.00 db, a mean of 5.77 db. The present figures

[8] The authors are indebted to Mr. E. J. Van Loon for making the blood-sugar and glycogen determinations.

being from a single animal, their agreement with the general means from Roentgenized dogs is obvious. *b*. We see also that the effect here is less for 125 ~ than thereabove.

Blood-sugar was determined finally in one dog 21 (each sample being drawn after an 8-hour fast) both before and after Roentgenization; on five successive days just prior to radiation, and on five days (fifth to ninth inclusive) after radiation, when the hearing-effect was maximal. From these figures, summarized in table 1, we observe that the customary gain in acuity is attended by a small but consistent hypoglycemia. The differences, in both hearing and blood-sugar, are statistically sound. The reduction of blood-sugar is indeed markedly less than followed direct injection of insulin in animal 20 above, whereas the hearing-effect is of the same order in magnitude. But the crucial question in each case is: what was the relative sugar-content *within the cochlea?* It is clear that the long latency of the x-ray effect affords ample time for the peri- and endo-lymph to attain complete glycose-equilibrium with the blood; whereas the 30-minute period between insulin-administration and hearing-tests in animal 20 may well have left the glucose-content of the cochlear lymph but little affected despite marked hypoglycemia in the blood. Indeed, one authority states[9] that a latent period of 40 to 90 minutes intervenes before a change in blood-sugar content "begins" to influence cerebrospinal fluid sugar. Hence it appears inevitable that peri- and endo-lymph were far indeed from sugar-equilibrium with the blood when the hearing-tests were made.

From the above evidence, it appears that the cellular structure of the hypophysis, after bombardment by a Roentgen-beam, passes through a period of degeneration and reconstruction, during which time the gland's normal functions are impaired. As compared with hypophysectomy, the symptoms are identical in kind but milder in degree: increased output of insulin, with concomitant reduction of sugar throughout the bodily fluids, including the cochlear lymph. Thus far we are on the solid ground of experimental knowledge. One question still remains: how does change in sugar-content of endo- and peri-lymph affect hearing? Two answers may be suggested. *a*. Low sugar lessens both density and viscosity of the

[9] S. Katzenelbogen, in a recent book, surveys and evaluates the factual material concerning the relation of sugar level in cerebrospinal fluid and in blood. To quote: "The existence of a quantitative relationship between blood sugar and C. S. F. sugar is no longer disputable. . . . Diversities in the blood sugar level are followed by corresponding changes in the sugar content in the C. S. F. However, there is always a certain latent period—from 40 to 90 minutes—before the change in the blood sugar content begins to influence the C. S. F. sugar. Moreover, the same studies demonstrate that the disturbed equilibrium between blood and C. S. F. sugar, induced by experimental hyper- or hypo-glycemia, may last 6 hours before it returns to its original level" (**15:** 149, 152).

cochlear fluids, which therefore offer less resistance by inertia to incoming vibrations than they normally do. Weaker sounds are able in consequence to activate the basilar membrane. *b*. Sugar, being a non-electrolyte, may so modify ionic conditions within the cochlea as to affect the magnitude of potentials generated within the hair-cells by an impressed oscillation. The first is necessarily true, the second may be an adjuvant factor.

SUMMARY AND CONCLUSIONS

1. The transient gain in acoustic sensitivity following x-radiation of the head, previously reported, is here confirmed for three frequencies (125, 1000 and 8000 cycles). Two objections to the validity of this effect are explored and answered. *a*. It can not be ascribed to increased random responsiveness of the conditioned limb, for two reasons. i. Spontaneous activity of the test-limb does *not* increase after Roentgenization. ii. Even when, for other causes, spontaneous activity of the test-limb does increase, the animal's acoustic thresholds, as measured by our methods, are *not* affected. *b*. It is proved that the dosages required to produce the hearing-effect are more than ample to penetrate the entire cranial content of a dog.

2. Both magnitude and duration of the effect remain constant throughout the range, 75 to 675 r-units; but its latency decreases as Roentgen-dosage increases.

3. The effect is slightly less at low frequencies (125 ˜) than at medial and high levels (1000 and 8000 ˜).

4. Two normal animals, when radiated on areas other than the head, showed no trace of the effect.

5. Two hypophysectomized dogs, when radiated, revealed no change in acoustic threshold; whence we see that the effect issues from the pituitary gland.

6. About 40 days after hypophysectomy, without any exposure to x-rays, the threshold of these two dogs declined and remained about 5 db below its level theretofore. From that phenomenon, combined with the other evidence, we infer a two-fold acoustic change: *a*, the true "x-ray effect," appearing within a few days, which is due to a mild and transient depression of sugar throughout bodily fluids, including peri- and endo-lymph; *b*, the "hypophysectomy effect" which first, like x-rays, produces a moderate hearing-gain and later, after 40 days, a second gain, symptomizing the continued depression of sugar to a more severe and chronic level.

7. A diabetic subject, and also a normal dog, showed increased acuity when blood-sugar was lowered with insulin and reduced sensitivity when blood-sugar was raised.

8. The sugar-level of an x-rayed animal was found to be subnormal during the period when its acoustic threshold was subnormal.

9. The mechanism of the above effects is briefly set forth.

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AN EXPERIMENTAL INDEX OF ERYTHROPOIETIC FUNCTION IN RABBITS

PHILIP L. KURTZ

From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital, and the Department of Medicine, Indiana University School of Medicine, Indianapolis

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The fact that the number of erythrocytes per cubic millimeter of blood in a normal animal at rest is such a constant figure is evidence of a sensitive physiological mechanism. The maintenance of the normal red blood cell level requires a certain definite bone marrow output of new cells because of the natural processes of red cell destruction. A relative estimate of this output may be made by the determination of the number of reticulocytes per cubic millimeter of blood; i.e., the absolute reticulocyte count.

The recognition of the bone marrow as a major organ of the body, as mentioned by Sabin and Doan (1), is fundamental. In studying bone marrow function with regard to erythrocyte output, the most promising approach with animals is the reticulocyte response to blood loss by hemorrhage. Steele (2) has shown that "the marrow pattern finds direct expression in terms of the cells of the blood." A. S. Gordon (3) points out the quantitative nature of reticulocyte responses to acute hemorrhages resulting in the loss of approximately the same per cent of red blood cells.

From the total blood volume, the red blood cell count and the absolute reticulocyte count, a provisional estimate will be made in this study of the daily normal red blood cell loss. A comparison will then be made of the reticulocytes produced per cubic centimeter of blood loss per day by natural processes with reticulocytes produced per cubic centimeter of blood loss per day by hemorrhage when the animals are subjected to small daily bleedings over a long period of time.

In order that this be of significance, it is important that averages of daily determinations be used to obliterate normal fluctuations and variations due to experimental errors. In addition, time must be allowed for an equilibrium to be established between bone marrow, blood depots, and peripheral blood in animals made anemic by chronic hemorrhage.

Let V equal the normal total volume of circulating blood in cubic centimeters.

Let R equal the normal absolute reticulocyte count in millions per cubic centimeter. (In the graph, cell counts are recorded as cells per cubic millimeter of blood, but in the text these figures are expressed as millions per cubic centimeter in order to facilitate calculations.)

Let E equal the normal erythrocyte count in millions per cubic centimeter.

Let L be an equivalent of red blood cell loss per day by natural processes of destruction expressed as cubic centimeters of blood having the normal number of red blood cells per cubic centimeter.

In order that any red blood cell level may be maintained, there must be as many new cells formed each day as old ones lost. Hence, the total blood volume in cubic centimeters multiplied by the number of reticulocytes per cubic centimeter should be equal to the equivalent of red blood

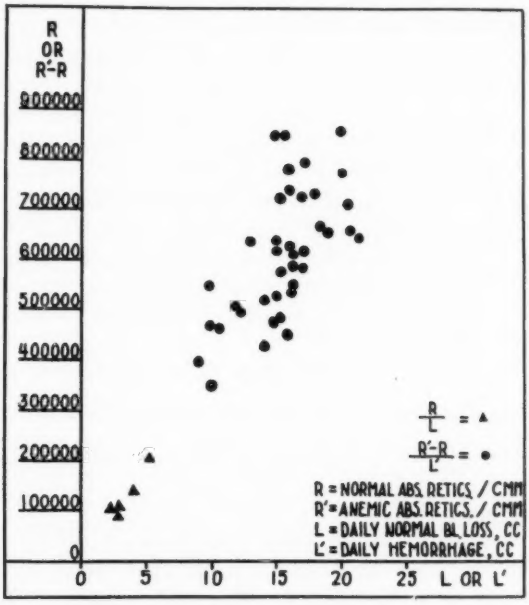

Fig. 1

cell loss per day expressed as cubic centimeters of blood, multiplied by the normal number of red blood cells per cubic centimeter:

i.e., $$V \times R = L \times E$$
also, $$R/L = E/V$$
and solving for L, $$L = VR/E$$

This is true if the absolute reticulocyte count represents the number of reticulocytes produced each 24 hours because L by definition is blood loss per 24 hours.

E, R and V were determined for five rabbits. E and R represent averages of daily counts for from four to six days. V is the blood volume

as determined on the last normal control day by the brilliant vital red method (4).

The values for L were calculated from the equation, $L = VR/E$. In figure 1, R is plotted against L to show the number of reticulocytes per cubic centimeter of blood produced in response to blood loss by natural processes of red cell destruction. The number of reticulocytes per cubic centimeter of blood for each cubic centimeter of blood loss per day is R divided by L and according to the equation, equals E divided by V. The average value for E/V for these five animals was 35,900 with upper and lower limits of 39,300 and 32,000 respectively. These five rabbits and seven others have been subjected to chronic hemorrhage. After a variable period of adjustment, red blood cell counts, reticulocyte determinations and bleedings were averaged for periods of nine days. The nine day period was chosen because it represents somewhat more than a recent estimate of the longevity of the circulating red blood cell in rabbits (5). Using the data so obtained an interesting relationship is to be noted.

Let R' represent the reticulocytes expressed in millions per cubic centimeter in the anemic animal, or in other words, the reticulocytes per cubic centimeter necessary to maintain the normal red blood cell level plus the reticulocytes per cubic centimeter produced in response to hemorrhage.

The reticulocytes per cubic centimeter produced in response to hemorrhage are represented by $R' - R$.

Let L' equal cubic centimeters of blood loss by hemorrhage per day. Then $\frac{R' - R}{L'}$ is the absolute reticulocyte increase in millions per cubic centimeter for each cubic centimeter of blood loss per day (abbreviated in the remainder of the paper, A. R. I. per cc.). This corresponds to R/L at the normal red blood cell level.

Averages were taken of red blood cell counts, absolute reticulocyte determinations, daily bleedings in cubic centimeters and A. R. I. per cc. for 39 periods of nine days, distributed among twelve rabbits. The A. R. I. per cc. did not vary uniformly with the red blood cell level when this level was above 3.13 million per cubic millimeter, nor with the amount of hemorrhage per day. The average A. R. I. per cc. of these 39 determinations was 38,700 with upper and lower limits of 51,000 and 30,000 respectively. This closely approximates the average value for E/V which was 35,900.

Charting $R' - R$ against L', figure 1 shows the A. R. I. per cc. to fall almost directly in line with the average R/L and, therefore, E/V. It is noted that the majority of the points fall not on the line but to one side or the other of it, suggesting that the A. R. I. per cc. is not a constant for all animals but subject to individual variation. There was indication that it varies inversely with the age and weight of the animal.

The equation $R/L = E/V$ was formulated for animals at the normal red blood cell level. L, by definition, is the cubic centimeters of blood loss per 24 hours; however, we are not certain that reticulocyte counts used for R represent the number of reticulocytes per cubic centimeter produced each 24 hours. The A. R. I. per cc. is $\frac{R' - R}{L'}$ and here it is known that L' is blood loss per 24 hours. Since $\frac{R' - R}{L'}$ gives an index so close to R/L, it would appear that if the reticulocyte response to blood loss by hemorrhage is comparable to the response to natural red blood cell destruction, R is the number of reticulocytes per cubic centimeter produced in 24 hours. Therefore, the number of red blood cells per cubic centimeter divided by the number of absolute reticulocytes per cubic centimeter is the length of life of the red blood cell in days.

If this is true, reticulocyte determinations take on added significance, although it must be remembered that counts and bleedings for periods of nine days were averaged and these averages used to determine the A. R. I. per cc. This obscures the actual daily variations.

For the twelve rabbits used in this experiment, the normal red blood cell count divided by the normal reticulocyte determinations gave an average of 42 days for the length of life of the red blood cell in the circulation, with upper and lower limits of 73 and 16 days respectively.

CONCLUSIONS

1. The number of red blood cells per cubic centimeter divided by the total blood volume in cubic centimeters (when these values are not rapidly fluctuating) suggests itself as an index of erythropoietic function. The figures so obtained in normal rabbits are remarkably close to the absolute reticulocyte increase per cubic centimeter of blood for each cubic centimeter of blood loss per day when the animals are subjected to chronic hemorrhage.

2. Further evidence is given to support the view that the reticulocyte response to blood loss is normally a quantitative one.

3. The average length of existence of the circulating erythrocyte in twelve rabbits was calculated to be 42 days.

I wish to express my thanks to Dr. G. F. Kempf and Dr. L. G. Zerfas for their suggestions and kind criticism.

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THE EFFECT OF ADVANCING AGE ON THE OXYGEN CONSUMPTION OF RATS

J. E. DAVIS

From the Lasker Foundation for Medical Research, University of Chicago

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This study was undertaken in order to determine the effect of advancing age on the oxygen consumption of rats. Benedict and MacLeod (1) made such a study using mostly different rats for the successive age groups and employing a technique that failed to eliminate activity and to permit discrimination among rates for waking, dozing and sleeping states. With the advantage of a technique that permitted such discrimination, it seemed advisable to repeat this work using the same rats for the successive determinations except as death diminished the number available. The results, therefore, constitute a life record, part of which, that for the immature rats, has already been published (2). This paper is concerned chiefly with the mature rats, but for the sake of completeness the whole record has been included in the tables and figures in order to facilitate comparison with the human situation.

The rat colony numbered 136 and was composed of two strains, a white, and a black and white, but, as the two strains showed little difference in oxygen consumption, this classification was not continued. They were kept on the same balanced diet throughout and in an environmental temperature of 25° to 28° except during the hot spells of 1934 and 1936 when that temperature was exceeded, and during which the most deaths occurred.

The method of determining the oxygen consumption has already been described in the introductory article (2). The amount of oxygen consumed was expressed in terms of liters per kilogram of body weight per 24 hours. Each determination was made after a fasting period of 16 to 21 hours. During a determination the rat was under a continual observation so that its state was recorded during every 5 minutes for which readings were made. Calculations were based only on those readings for which a dozing or a sleeping state was recorded. The sleeping state was characterized by continuous sleep, and gave the lowest rate observed. The dozing state was characterized by the rat opening and closing its eyes every few seconds and seeming to be on the verge of sleeping without actually sleeping, and gave a rate 10 to 15 per cent higher than that for the sleeping

state. The oxygen consumed by one rat during 8 consecutive 5-minute periods was as follows: 25.0, 24.5, 25.0, 24.5, 23.0, 22.0 cc., respectively, for the dozing periods; and 21.5, 21.0, 21.5 cc., respectively, for the sleeping periods. The values obtained for the last 2 periods of the dozing state probably represented a state intermediate between dozing and sleeping. The inclusion or exclusion of these values would result in a difference of approximately 3 per cent in the calculation of the rate. In all cases the difficulty of making a reading correct to within 0.5 cc. introduced an error of 2 to 3 per cent. In the case of the more easily recognized sleeping state this was likely to be the extent of the error, but in the more difficultly recognized dozing state the probability of including both errors was much greater.

TABLE 1

Life record of average oxygen consumption of 136 rats

AGE	LITERS OF OXYGEN CONSUMED PER KILOGRAM OF BODY WEIGHT PER 24 HOURS							
	Males			Females				
					Dozing		Sleeping	
	Number	Dozing	Sleeping	Number	Estrous	Non-estrous	Estrous	Non-estrous
months								
1	66	39.5	32.5	56		39.6		33.2
2	71	39.1	32.1	60		38.5		31.8
3	68	31.5	26.8	60		30.9		26.0
4	45	25.5	21.6	55		25.6		21.2
6	70	24.0	20.7	53	25.2	23.1	22.9	20.3
10	64	22.8	20.0	50	24.0	22.0	21.9	19.6
14	47	21.8	19.0	34	23.0	21.0	21.0	18.5
18	30	20.0	17.5	11	22.0	19.1	19.5	17.2
22	16	19.0	16.8	23	21.0	18.1	18.5	16.2
26	14	18.4	15.1	10	19.7	17.1	17.0	14.9
30	10	16.8	14.5	10	18.0	16.0	15.7	14.3

Beginning with the sixth month the females were examined for estrus determined by the presence of cornified cells, which were found even in some of advanced age. Such rats were classified as in estrus as was also done by Slonaker (3) though he found that they rarely, if ever, became pregnant. The determinations for the females, therefore, included a dozing and a sleeping rate for both the estrous and non-estrous state, whereas those for the males included only a dozing and a sleeping rate. The averages of these 2 determinations for the males and the 4 for the females of the various age groups are shown in table 1. For the sake of completeness the first month's averages have also been included though they were omitted in the earlier paper (2) because they were based on determinations following breast feeding and might not, therefore, be consistent

with those following feeding on the later diet. In the earlier paper dealing with the immature age groups it was shown that the probable error due to inadequate sampling was insignificant. Similar results have not been included in this table because the number of rats in some of the later age groups seemed too small to justify such procedure. Statistical calculations, however, show the same results as before for the larger adult age groups, and, since it may be seen from figure 1 that the decrease in their oxygen consumption continued at about the same rate through the later smaller age groups as through the earlier larger groups, it seems probable that the less adequate sampling of the smaller groups had not introduced a significant error. It seems, therefore, justified to observe that the oxygen consumption of these rats decreased with advancing age, and that the rate of decrease was similar for the dozing and sleeping states of the males and of the estrous and non-estrous females.

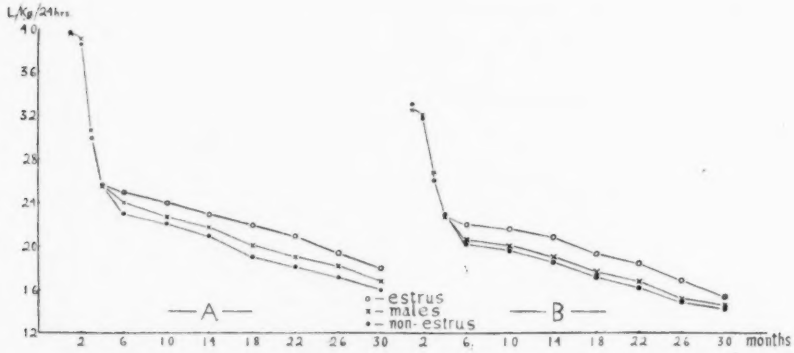

Fig. 1. Life record of average oxygen consumption of 136 rats: A, dozing; B, sleeping.

The individual determinations were too numerous to be included in table 1. In the case of the adult females these values group themselves into high and low, corresponding, respectively, to the estrous and non-estrous states. The possibility of a similar grouping of values for the third month suggests the probability that estrus was the discriminating factor there too. On the same basis the values of the fourth month were with 2 exceptions high enough to be considered representative of the estrous state.

It may be seen from figure 1 that the estrous rate was higher and the non-estrous rate somewhat lower than the rate for males for both the dozing and sleeping states. It seems especially noteworthy that so little difference occurred between the sleeping rate of the male and of the non-estrous female. Though the difference was about the same order as the

experimental error, its consistency throughout would suggest that this difference, however small, was probably real. However, such a difference might be the result of a carry-over from a previous state, and had the sleeping state lasted for hours instead of minutes, the values might have been more nearly identical. It was observed that the females were more restless in estrus and that fewer sleeping rates were obtained for the estrous than the non-estrous state. It was also observed that the males were not as reconcilable to the conditions of a determination as were the females. It was further observed that there was great variation in the

TABLE 2*

Life record of average heat production of 136 rats

AGE	CALORIES PRODUCED PER SQUARE METER OF SURFACE AREA PER HOUR											
	Males				Females							
					Dozing				Sleeping			
	Dozing	Weight	Sleeping	Weight	Estrous	Weight	Non-estrous	Weight	Estrous	Weight	Non-estrous	Weight
months		*grams*		*grams*		*grams*		*grams*		*grams*		*grams*
1	33.2	57	27.2	57			33.0	56			27.7	56
2	43.0	130	35.4	130			40.5	113			33.5	113
3	38.7	179	32.8	179			35.8	151			30.1	151
4	33.8	225	28.6	225			31.2	175			26.0	180
6	33.8	272	29.2	273	32.1	201	29.5	202	29.3	202	26.0	202
10	33.3	302	29.1	298	31.4	218	29.1	225	28.9	225	25.9	225
14	32.9	331	28.9	340	30.7	231	28.8	249	28.8	250	25.2	246
18	31.0	360	27.6	380	30.2	249	26.8	270	27.3	268	24.2	269
22	30.3	394	27.0	401	29.7	273	26.1	291	26.6	289	23.3	290
26	29.5	398	24.6	423	28.6	297	25.2	311	25.0	312	22.0	313
30	27.1	410	24.0	439	26.7	315	24.2	334	23.7	332	21.6	333

* The oxygen consumption values of the first table were converted into heat production values in this table on the following bases: 1, that the consumption of 1 liter of oxygen resulted in a heat production of 4.7 calories; and 2, that the body surface in square centimeters is equal to 9 times the two-thirds power of the body weight in grams.

oxygen consumption of some of the males and females when awake, even though no movement was perceptible. These considerations suggest the probability that the differences observed between the rates of the male and the female, and of the estrous and non-estrous female, might have been due to the effect of some such intangible factors as fear, tension, alertness, etc., imposed upon an otherwise fundamentally equal rate.

For the sake of comparison the oxygen consumption values of table 1 have been converted into heat production values in table 2. The converted values also show a generally decreased heat production as age

advances. There is, however, a noticeable discrepancy in the comparatively lower value for the first month on the basis of surface area than on the basis of weight. The explanation would seem to lie in the probability that the heat production at this age depended more on growth and weight than on surface area.

The results of table 2 are not in accord with those obtained by Benedict and MacLeod (4) who found that the heat production of rats increased

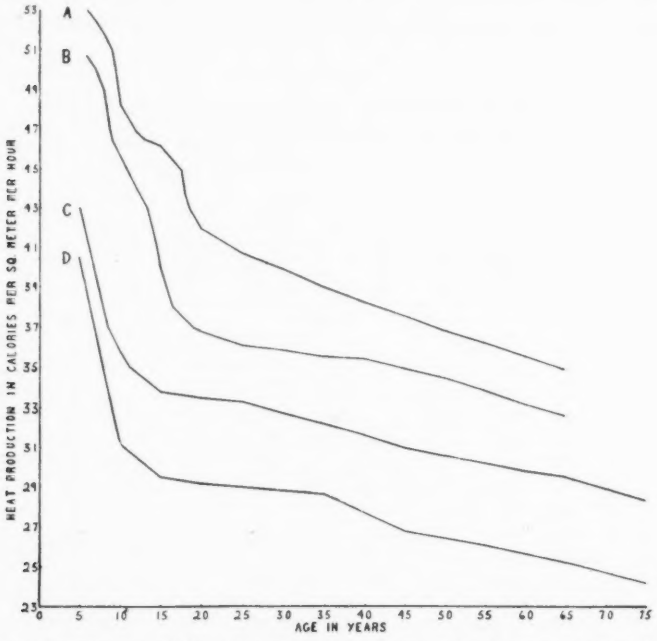

Fig. 2. Comparison of the life heat production records of men and rats: A and B being smoothed curves for men and women, respectively, based on the Mayo Foundation Standards; C and D being similar curves for the dozing male and non-estrous female rats of table 2, made comparable with curves A and B by multiplying the rat ages by 30.

with advancing age. In a more recent work, information of which was received by private correspondence, Benedict and Sherman (5) corroborate in the main the earlier findings of Benedict and MacLeod. In both studies, however, Benedict and his associates used a technique that did not discriminate between waking and sleeping rates and, therefore, did not eliminate the possibility that the increase they observed might have been due to a change from a younger state in which sleep predominated to an older one in which wakefulness predominated.

The results of table 2 are more in accord with those obtained for man by Boothby, Berkson and Dunn (6). This is shown in figure 2 where the smoothed curves A and B for men and women, respectively, are compared with the smoothed curves C and D for dozing male and non-estrous female rats, respectively. To make the rat curves comparable with those for men and women, the rat ages have been multiplied by 30 since it is generally conceded that one day of a rat's life is approximately equivalent to a month of man's life. On this basis the two sets of curves show a striking similarity which seems to indicate that rats and men differ but little both in the per unit amount of their heat production and in the rate at which it decreases with advancing age.

The results of this study may be summarized as follows:

1. The average oxygen consumption rate of the 136 rats studied showed a continual decrease throughout life, rapid during the first 4 months, more gradual thereafter.

2. The method used enabled discrimination between rates for dozing and sleeping states of the males and of the estrous and non-estrous females, from which there appeared to be little difference between the sleeping rates of the males and the non-estrous females.

3. There appears to be a close relationship between the heat production of rats and of men both in its per unit amount and in its rate of decrease with advancing age.

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OXIDIZING ENZYMES IN BRAIN EXTRACTS[1]

M. B. COHEN AND R. W. GERARD

From the Department of Physiology, University of Chicago

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The physiological and pathological activity of the central nervous system is dependent in high degree on its metabolic processes and so on its enzyme complement. Attempts at analysis, however, result in considerable confusion, due to the complexity of organization and to local metabolic differences in the whole organ. A simpler system, more favorable for study though less complete, results from minimizing structural differences and destroying some enzyme systems. This has been achieved by preparing a homogeneous colloidal suspension of brain in water. Such a sol has a small residual respiration, limited apparently by available substrate, and contains many of the enzymes present in whole brain, although others, particularly those involved in carbohydrate oxidation, are inactivated. The sol, and a protein-lipoid fraction obtained from it by isoelectric precipitation, carry concentrated systems for oxidizing succinic acid and paraphenylenediamine. A partial analysis of the enzyme mechanisms present is described below.

METHODS. A rabbit was stunned by a blow, the brain quickly removed, pia and blood peeled off, and the desired tissue weighed and kept on ice. Only cerebral cortex and cerebellum were used, since the underlying tissues contain a large proportion of white matter which is difficult to cytolyze. When cool, the tissue was minced, then ground, and finally dispersed with nine volumes of iced distilled water. The suspension was centrifuged 20 minutes and the supernatant solution pipetted off. About $\frac{3}{4}$ of the total volume was used. This supernatant colloidal solution (referred to in subsequent pages as "the sol") was seen under the microscope to be cell-free and virtually structureless except for dispersed particles, although it was cloudy in bulk. Further, filtration through a porcelain candle had little effect on its properties. No material settled out on standing.

Other preparations were also used: 1, the washed residue obtained on centrifugation, containing mostly cell debris and white matter; 2, a protein-lipoid fraction prepared from the solution by isoelectric precipitation (at

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about pH 4.6), washing and resolution in dilute alkali; and 3, dehydrated or dehydrated and defatted preparations of the precipitate.

Oxygen consumption was measured in modified Warburg manometers (Gerard, 1931) at 37°C. The total volume of fluid in each chamber was always 1.0 cc., 0.1 cc. of this being N/2 NaOH and the rest either brain extract or extract plus the desired reagent. The preparations were unbuffered unless otherwise stated.

RESULTS. I. *Respiration of the sol.* A. *Residual respiration* of the sol was comparatively high the first hour, but fell gradually to nil by the 4th to 6th, presumably with the exhaustion of available substrate. Typical Q_{O_2} values (cubic millimeters oxygen consumed per cubic centimeter brain

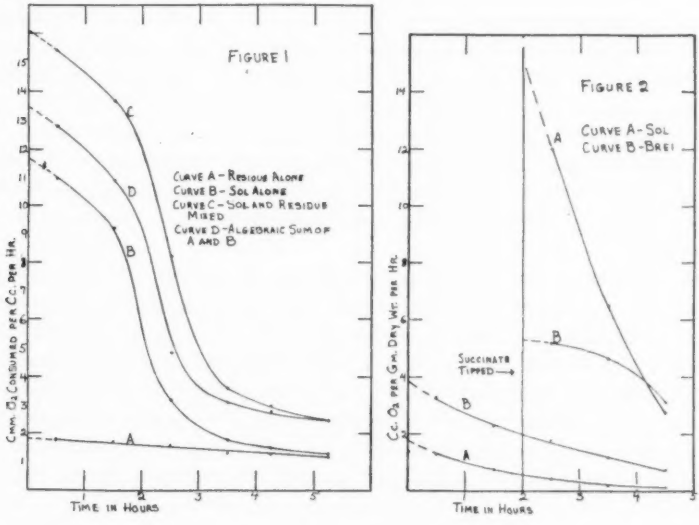
FIGURE 1
CURVE A - RESIDUE ALONE
CURVE B - SOL ALONE
CURVE C - SOL AND RESIDUE MIXED
CURVE D - ALGEBRAIC SUM OF A AND B
CMM. O2 CONSUMED PER CC. PER HR.
TIME IN HOURS
FIGURE 2
CURVE A - SOL
CURVE B - BREI
SUCCINATE TIPPED
CC. O2 PER GM. DRY WT. PER HR.
TIME IN HOURS

solution per hour) are shown in figure 1. The total oxygen consumption for the first three hours averaged 18.1 in all experiments (50). Since each preparation was homogeneous, duplicate manometers gave excellent agreement, but rates for different brain sols varied from 15 to 30. The 3 hour average per milligram dry weight was 0.35 to 0.5 cmm. O_2 per hour (compare with 3 to 5 for minced brain), that for the first hour being 1.0. For intact brain (Brookens, Ectors and Gerard, 1936) initial values per milligram dry weight may be as high as 20, though in vitro average values are 5 to 10 during the first hour.

This residual respiration falls as the sol ages, and even at 1 to 2°C. disappears in 1 to 2 weeks though 85 per cent remains after 3 days. The respiration is partly heat stable, over $\frac{1}{3}$ remaining after a half-hour at 60°C.; lactate oxidation is likewise partially heat stable (see later).

The R.Q. of the sol is 0.48 (compare with an R.Q. of 1.0 for whole brain, Dickens, 1936). Such a low value could not be associated with the complete oxidation of carbohydrate, protein or lipoid. It suggests, rather, that dehydrogenations are not carried to completion, so that little CO_2 results.

What substrate is oxidized during residual respiration, which is quite constant from one brain to another, is of interest; the more so since it is not one of the carbohydrate derivatives responsible for the major part of the respiration of intact brain (e.g., Holmes, 1932). Sodium monoiodoacetate inhibits glucose oxidation between the triose-phosphate and pyruvic acid stages (Meyerhof, 1933) or even earlier and should, therefore, decrease residual respiration to the extent that glucose or hexose-phosphate is involved. The observed depression, with M/100 iodoacetate, is only 10 per cent (compared with 87 per cent in brain slices), so that no considerable carbohydrate oxidation is occurring. Further, lactate added to sol is oxidized, but glucose hardly at all. According to Quastel and Wheatley (1932), many narcotics inhibit the dehydrogenation of glucose, lactate and pyruvate by brain tissue, yet urethane in the concentration they used (0.25 per cent) gave no significant inhibition and M/100 sodium arsenite, another dehydrogenase inhibitor, decreased the oxygen consumption of the sol only 6 per cent (1 expt.), while that of brain brei was decreased by 73 per cent.

Cyanide, by contrast, cuts out two-thirds (63 per cent) of the residual respiration, presumably by action on the indophenol oxidase. This is true for M/100 cyanide; a more dilute solution, M/1000, increases oxidations by as much as 100 per cent in some experiments, 31 per cent on the average. Apparently at sufficient dilution cyanide accelerates certain oxidations more than it inhibits others. Cyanide-hematin is a powerful catalyst for lipoid oxidations (Wright and Van Alstyne, 1931) and cyanide increases the oxidation of methyl-glyoxal (Meyerhof, 1925; Ariyama, 1928; see below). It prevents the inhibitory action of pyruvic acid on lactate oxidation (Green and Brosteaux, 1936) and may itself undergo a coupled oxidation with glucose in alkaline solution (Harned and Deere, 1934). The accelerating action of cyanide on the respiration of *Chlorella* in the resting state and its inhibiting effect on the extra respiration in nutrient solutions will be recalled (Emerson, 1927). An attempt to demonstrate oxidation of sol lipoids in the presence of cyanide by a change in iodine number was unsuccessful.

To eliminate completely larger structural elements, the sol was filtered through a porcelain candle. This decreases oxygen consumption at most by one-fifth, and even this loss may have been due to a rise in temperature during filtration. The R.Q. and substrate oxidizing ability of the filtered material (so far as tested) are unchanged. The enzyme reactions of the sol are, therefore, essentially independent of organized structure. The

indophenol oxidase and probably other enzymes are associated with fairly large particles since they can be centrifuged out of solution with even moderately high speeds (Ogston and Green, 1935b; Hoerr, personal communication); and for brain sol also sufficient centrifuging reduces both the residual respiration and the oxidation of added succinate and paraphenylenediamine. The particles in this case must be under 0.02 to 0.03μ since little or no enzyme loss resulted from filtration through porcelain of such pore size.

The washed residue from the original centrifugation, with almost no respiration of its own, may still double the respiration of the sol on remixing with it. The average increase is 20 per cent (fig. 1) for unfiltered sol, 50 per cent after filtration, and the excess oxygen consumption is over in four hours. On remixing the washed residue with its washings there was no such effect. The accelerating influence of the debris is not related to its contributing "structure" to the mixture since no increase of sol respiration results from addition of inert colloids, such as acacia or albumin. Of course a specific active adsorption compound might be formed from moieties in the separate fractions; and it is interesting that urethane (0.25 per cent), which presumably acts by adsorption, entirely abolishes the extra respiration of mixed sol and residue while not affecting their separate respirations.

The extra respiration of the mixture is partly due to the oxidation of additional substrate, for not only is the rate greater but the total oxygen consumed, dependent on available substrate, is also increased by 20 per cent. It is striking that added lactate likewise increases the respiration of the mixture one-fourth more than it increases the sum of the respirations of sol and residue treated separately. Further factors are important, however, since mixing solution and residue after the oxygen consumption of each has ceased does not restore the respiration of the mixture as it should if additional substance can be burned by it.

B. *The effect of pH.* The pH of the solution immediately after preparation ranges from 6.7 to 7.0 from one brain to another, and is maintained during several days on ice, or throughout an experiment at 37°C. Residual respiration is not sensitive to moderate pH changes, for a series of 20 preliminary experiments showed this to be greatest at pH 7.6 and only 6 to 8 per cent less at pH 7.2 or 8.0. Addition of 1/10 volume of M/15 phosphate buffer at pH 7.4 does not noticeably affect the residual respiration of the sol or its oxidation of any substrate except methyl glyoxal. It may, moreover, be acidified to pH 3.8 and reneutralized without significant decrease of oxygen consumption.

C. *Oxidation of substrates.* The rate (and amount) of oxygen consumption of the sol might be limited by the amount of enzyme or of substrate present. In the latter case, addition of an appropriate substrate should

increase respiration. The following were tried in M/100 and sometimes M/1000 final concentration: glucose, fructose, sodium lactate, glycerophosphate, pyruvate, succinate, citrate and oleate, methyl glyoxal, glycine and paraphenylenediamine. Of these, sodium succinate, sodium glycerophosphate, sodium lactate, fructose, methyl glyoxal and paraphenylenediamine are oxidized. Sodium oleate and citrate inhibit the residual respiration by 30 to 50 per cent. Results are summarized in table 1.

Glucose. In a large series of experiments, no increased oxygen consumption could be demonstrated on adding M/100 or M/1000 glucose. An essential enzyme seems peculiarly sensitive to destruction of cell structure, for glucose addition increases the respiration of chopped but not cytolysed brain about 40 per cent. Progressive dilution of brain brei with

TABLE 1

Effect of substrates on oxygen consumption of the solution

SUBSTRATE ADDED	NUMBER OF EXPERIMENTS	O_2 CONSUMPTION IN PER CENT OF SOL ALONE	
		first hour	*average first 3 hours*
Glucose, M/100	10	93	94
Lactate, M/100	5	178	150
Citrate, M/100	2	70	74
Oleate, M/100	4	47	54
Oleate, M/100 + $CaCl_2$, M/100	2	75	71
Fructose, M/100	2	125	129
Pyruvate, M/100	2	110	95
P-phenylenediamine, M/100	2	1450	1220
Succinate, M/100	5	2150	1570
Methyl glyoxal, M/100, unbuffered	4	115	111
Methyl glyoxal, M/100, buffered	2	129	125
Glycerophosphate, M/100	2	162	136

water does not stop glucose oxidation until a dilution (1:14) greater than that in the sol is reached (table 2), presumably because the inner cells of a clump are protected against cytolysis. Brei with water added, which oxidizes glucose, and the uncentrifuged suspension, which does not, differ as to the degree of cytolysis and the amount of dilution of cell protoplasm consequent to cytolysis. The former factor seems the more important since, when complete cytolysis is assured by thoroughly grinding the chopped brain, a dilution of less than 1:2 entirely eliminates glucose oxidation. This can hardly entail a serious loss of coenzyme, and active glucose dehydrogenases have been obtained from liver (Harrison, 1931) and blood cells (Warburg and Christian, 1931) after destroying cell structure. Further, a loss of the oxidase-cytochrome system, if involved at all, is not important, since methylene blue does not restore glucose oxidation.

Presumably the dehydrogenase has somehow been inactivated by our procedure of water cytolysis.

Other workers have noted that dilution per se may cause diminution of respiration. Thus Warburg (1911) found that a concentrated suspension of laked red cells uses 60 to 75 per cent more oxygen than intact cells, but that dilution causes a rapid decrease in oxygen usage, and Krebs (1935) confirmed this for a suspension of kidney brei. He suggested that systems which lose activity on dilution involve reactions of ternary or higher orders, since dilution rapidly decreases the chance of multiple collision. In the present system, except with glucose, dilution has little effect, so either binary reactions are involved or more than one reactant is carried on the same colloidal particle. This seems to be the case for the oxidase-cytochrome-succinic-dehydrase system.

Lactate is rapidly oxidized by the sol, the extra oxygen consumption (80 per cent) being nearly completed in an hour. It is also oxidized by

TABLE 2

Effect of water dilution on oxidation of glucose by brain brei

BREI	WATER	RINGER	Q_{O_2} WITHOUT GLUCOSE	Q_{O_2} WITH GLUCOSE	PER CENT INCREASE
gram.	*cc.*	*cc.*			
0.05	0	0.75	403	623	43
0.05	0.2	0.55	444	577	30
0.05	0.3	0.45	438	680	55
0.05	0.4	0.35	452	483	7
0.05	0.5	0.25	389	452	16
0.05	0.6	0.15	299	325	9
0.05	0.7	0.05	166	203	22

the washed residue, increasing respiration about 200 per cent. Added to a mixture of sol and residue, lactate causes an extra respiration greater than the sum of the separate increases. Thus, with lactate, the oxygen consumption of sol and residue run separately totals 22.2 cmm. for the first hour, while that of the mixture is 28.4, an increase of 28 per cent. Recombination of dehydrogenase and coenzyme, partially separated in residue and sol, could account for this increase.

The oxidation of lactate is not entirely abolished by heating, even to boiling. The oxygen uptake of heated sol plus lactate (13.5 cmm.) is 3 times that of the heated sol alone (4.5 cmm.) and over half that of the unheated sol plus lactate (20.2 cmm.). What the heat stable mechanism for lactate oxidation may be is unknown—no such effect was noted with any other substrate.

Methyl-glyoxal is oxidized to a moderate extent, the average increase in respiration being 11 per cent for 3 hours. As it oxidizes, acid is formed,

sufficient to precipitate the sol proteins after 1 to 2 hours. Addition of buffer prevents this precipitation, and oxygen consumption is then increased by 25 per cent. The comparatively small amount of oxygen consumed by the methyl-glyoxal (25 cmm. or 1μM) would not correspond to enough acid production to precipitate the proteins, which requires about 8μM of HCl. Probably, therefore, methyl-glyoxal is undergoing a reaction involving acid production without oxygen consumption, as in dismutation to lactic acid.

Sodium succinate and p-phenylenediamine increase the residual respiration markedly, 20 and 14 times respectively for the first hour after addition. Succinate is oxidized so vigorously that, in M/100 concentration, oxidation

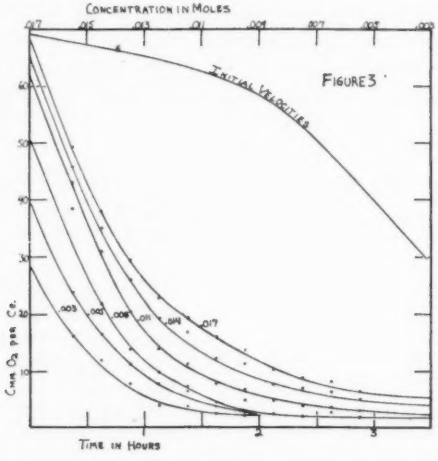

Fig. 3. Oxygen consumption of the sol in the presence of succinate in 0.003 to 0.017 M. concentration. Top curve represents initial oxygen consumption rates plotted against molar concentration.

is ended in 2 to 3 hours. Upon addition of more substrate, the oxygen consumption resumes nearly its original high rate. These substrates are also oxidized (though more slowly) by a sol that has been kept in the ice-box until its residual respiration is zero.

Although the oxidation by tissues and tissue extracts of succinate and p-phenylenediamine are commonly associated quantitatively (Thunberg, 1930; Quastel and Wheatley, 1932), the enzyme systems involved are not identical. Thus, Quastel and Wheatley (1931) have found that malonate inhibits the oxidation of succinate but not of p-phenylenediamine by brain slices, presumably by competing for the succinic dehydrogenase. Similarly, in the present brain sol malonate inhibited succinate oxidation, reducing to $\frac{1}{2}$ or $\frac{1}{3}$ the extra oxygen consumption due to succinate alone,

but had no effect on that of p-phenylenediamine. P-phenylenediamine is supposed to be oxidized by the Atmungsferment directly, while succinate oxidation depends on this plus a special dehydrogenase at least. Results with cyanide and methylene blue (see below) also point in this direction. Ogston and Green (1935a) offer evidence that the cytochrome-indophenol oxidase system catalyzes the reaction of succinic acid and its dehydrogenase with molecular oxygen and that even for p-phenylenediamine oxidation cytochrome is required in addition to the oxidase.

The respiration of chopped brain (50 mgm.) is increased 3 to 4 times in the first hour after adding sodium succinate (0.1 cc. M/10); that of the sol (1 cc.) 20 times (fig. 2). Succinate oxidation by the sol is almost complete in 2 hours, when 111 cmm. O_2 have been consumed, while in the same time the brei has consumed only 98 cmm. The extra oxygen used (above the normal respiration without substrate) per milligram dry weight of chopped brain and of sol respectively is 6.8 and 17.8 cc. for the first 2 hours; and the sol excess is even more striking for the first 30 minutes, during which the amounts are 2.4 for brei, 6.7 for sol. The sol is able, therefore, per unit dry weight, to oxidize succinate almost three times as rapidly as the brain from which it was prepared. Whether this represents a true concentration of enzyme, better diffusion relations in the sol, or loss of inhibitors present in whole brain (a "sparing" action of lactate has been reported, Quastel and Wheatley, 1932), has not been established.

At 37°C. and 750 mm. Hg, 125 cmm. of oxygen should oxidize 0.1 cc. M/10 sodium succinate to fumarate. In some experiments, however, the actual oxygen consumed exceeded this by as much as 60 to 65 cmm. Therefore, some of the oxygen consumed is used either to further oxidize the fumarate, or is being diverted to another substrate (vide St. Gyorgyi's theory of the carrier rôle of succinate-fumarate). The oxygen consumption never reached 250 cmm. (one molecule of oxygen per molecule of succinate). The R.Q. for the oxidation of succinate is zero—a slight CO_2 production (7 cmm. CO_2 to 192 cmm. O_2) is due to the residual respiration itself—and, of course, the transformation of succinate to fumarate does not involve the liberation of CO_2. The other oxidations, by the excess oxygen not accounted for by this reaction, must also stop before CO_2 is formed.

Oxidation rates were measured at succinate concentrations from 0.0009 M to 0.11 M in an effort to determine a velocity constant. The curves obtained have roughly the same slope between 0.003 M and 0.017 M (see fig. 3) but, assuming either one molecule or one atom of oxygen is used per molecule of succinate, calculations do not yield a constant.

Glycerophosphate is not oxidized as rapidly as the above substrates, but increases sol oxygen consumption 62 per cent for the first hour, 36 per cent for the first three. Green (1936) has found the glycerophosphate de-

hydrase more concentrated in brain than in other tissues. Its activity is enhanced by cyanide and sodazide, depressed by narcotics, and the main oxidation product is glyceraldehydephosphate.

Sodium citrate and *oleate* inhibit respiration, possibly by removing calcium ion; and addition of equivalent amounts of $CaCl_2$ partially restores oxidations. Calcium ion thus seems to influence the residual oxidations of brain sol in the reverse direction to oxidations in peripheral nerve (Chang, Shaffer and Gerard, 1935) or brain slices (Dickens and Greville, 1935).

D. *Effect of cyanide.* The respiration of sol alone is inhibited by M/100 and accelerated by M/1000 cyanide, but the extra oxygen consumption in the presence of substrates is uniformly inhibited. Methyl-glyoxal is an exception, its oxidation by sol or by chopped brain being accelerated 4 to 5 times (table 3). A similar influence of cyanide on methylglyoxal oxidation

TABLE 3

Effect of cyanide on the oxidation of substrates by the sol

SUBSTRATE ADDED	NUMBER OF EXPERIMENTS	OXYGEN CONSUMPTION IN PER CENT*
None	5	37
M/100 methyl glyoxal	2	529
M/100 Na succinate	3	10
M/100 Na succinate + 0.01 per cent methylene blue	3	18
M/100 p-phenylenediamine	2	4
M/100 p-phenylenediamine + 0.01 per cent methylene blue	2	3

* Values expressed in per cent of the oxygen consumption of the sol plus substrate without cyanide.

was reported by Meyerhof (1925) and confirmed by Ariyama (1928), who found the glyoxal was converted quantitatively to an unidentified volatile acid. Brain sol, however, almost doubles the oxygen consumption of a cyanide and glyoxal mixture, so that enzyme activity is also enhanced.

The oxidation of succinate is inhibited almost completely (92 per cent) by M/100 cyanide, and methylene blue (0.01 per cent) is able to restore it to one-fifth of normal. P-phenylenediamine oxidation, on the other hand, is completely inhibited by cyanide, and no restoration can be obtained with methylene blue. These results offer further evidence of two different oxidizing systems. Succinic acid presumably requires activation by a dehydrogenase as well as the presence of oxidized cytochrome before being oxidized, while p-phenylenediamine requires no special activation in the presence of the oxidase system (Keilin, 1929). Cyanide inhibits the oxidation of both compounds by preventing the oxidation of cytochrome. Methylene blue substitutes for the cyanide inhibited oxidase system and

completes the oxidation of succinate activated by its dehydrase (Gerard, 1932). Active succinate has a lower redox potential (-0.437, pH 7) than the p-phenylenediamine molecule (estimated to be about $+0.36$) so that methylene blue, whose potential is $+0.011$, can oxidize only the former.

II. *Respiration of the centrifuge residue.* This residue, about $\frac{1}{3}$ by volume of the original suspension and consisting of a semi-solid mass of cell debris, membranes, blood vessels, uncytolyzed nerve fibers, etc., consumes oxygen 2 to 3 times as rapidly as does the sol. The rate falls to zero after 3 or 4 washings, but the washed residue is still able to oxidize succinate and p-phenylenediamine rapidly, lactate to a lesser extent (18 cmm. O_2 per cc. in 3 hours) and glucose not at all. With succinate, 128 cmm. O_2 per cc. are consumed by the residue during the first hour, which is more than that for an equal volume of sol but considerably less per mgm. dry weight (12 cc. for sol; 3.5 for residue). This is true also for the oxidation of p-phenylenediamine.

III. *The isoelectric precipitate.* When acid is added to the sol, a precipitate of protein and lipoid components forms. It is most abundant at pH 4.6 and is partially redissolved in more acid or alkaline media. When redissolved in water and neutralized to litmus, this material still has a residual oxygen consumption $\frac{1}{2}$ to $\frac{1}{3}$ that of the original sol.[2]

The redissolved precipitate oxidizes succinate and p-phenylenediamine as rapidly as does the original sol, whereas the supernatant solution does not consume oxygen spontaneously or in the presence of any substrate tried. All activity, therefore, is in the precipitate. The decrease in residual respiration is not due to acid destruction of the enzyme, since if the solution is brought to pH 3.8 and reneutralized without separating it into fractions no decrease results. Substrate loss into the supernatant solution when the enzyme is precipitated probably accounts for the diminution.

The residual respiration of the precipitate is greatest on precipitation between pH 5.5 and 4.3. Below 4.3, although the volume of precipitate is still large, respiration is much smaller, and above 5.5 little precipitate is formed. The residual activity can be decreased by repeatedly washing the precipitate with acidified water or, less readily, with acidified phosphate solution, though the volume of the precipitate is little diminished. Two washings with water or three with phosphate are sufficient to abolish nearly all residual oxygen consumption.

Since succinate is oxidized by the precipitate, per milligram dry weight,

[2] It is worth noting that neutral red inhibits this respiration completely. A long series of experiments in which this dye was used as an indicator was thereby invalidated. It was later found that neutral red also inhibits the respiration of the sol, as well as the excess oxygen consumption produced by the addition of succinate. We are indebted to Dr. D. R. Briggs for first preparing the isoelectric precipitate.

more rapidly than by the solution, concentration of the succinate enzyme by fractional acid precipitation was attempted. Successive fractions were precipitated by small increments of acid and separately removed. The most active fraction (though not the greatest in bulk), obtained at pH 4.6 to 5.0, oxidizes succinate 2 to 3 times as rapidly as the original sol and 6 to 8 times as rapidly as brain brei. The purification of the enzyme is being pursued.

Discussion. When brain is cytolyzed and extracted with a large volume of water, the solid debris continues to consume oxygen, indicating retention of substrates and catalytic systems. After washing, this disappears due to substrate loss, since added lactate, succinate and p-phenylenediamine are still oxidized. The catalysts are retained, including the soluble coenzyme for lactate oxidation. Probably some cells have not been fully cytolyzed even by this drastic handling.

The colloidal tissue extract also contains complete oxidation systems for, at least, lactate, methyl glyoxal, α glycerophosphate, succinate and p-phenylenediamine, and continues to use oxygen until substrate is gone. The residual respiration is not inhibited by moderate concentrations of cyanide, though it is by M/100 cyanide, nor by narcotics or arsenite, nor by iodoacetate—though respiration of intact brain is depressed by all these agents in the same concentrations. Glucose can hardly be the substrate, since the glucose dehydrogenase system is quickly inactivated by cell destruction and dilution, since iodoacetate does not inhibit, and since, finally, glucose is probably all changed to lactic acid during the preparation of the brain fractions. Succinate oxidation should be inhibited by arsenite and urethane, and it is quite possible that stronger concentrations of these agents, as of cyanide, would inhibit, for intact cells are commonly more sensitive to inhibitors than are their oxidizing systems when reconstructed in vitro. But added succinate is so rapidly oxidized that any originally present could hardly account for the feeble prolonged residual respiration. This most likely depends on lactate, which is present in significant amounts and which is slowly oxidized when added. Further, on remixing residue and sol the oxygen consumption exceeds the sum of the two separately and the same excess is found for the increased respiration due to lactate addition. Whether this excess is due to remixing dehydrogenase and coenzyme, largely separated by extraction, or improved concentration relations of the reactants (e.g., if most of the dehydrogenase remains behind while most of the lactate and carrier are extracted) we cannot say. Structural factors do not seem to be concerned.

The lactate oxidation is, further, incomplete (R.Q. = 0.5) and somewhat anomalous in that it is in part thermostable (again like the residual respiration). This is being further explored.

The ready oxidation of added succinate demonstrates the presence of

this dehydrogenase and the indophenol oxidase-cytochrome system. Oxidation of succinate yields no CO_2, yet considerably more oxygen is used than required to change it all to fumarate. This extra oxygen could not all be accounted for by other substrates reducing fumaric (or oxalacetic) acid, as in Szent-Gyorgyi's (1936) scheme, since the total residual oxygen consumption (20 cmm. O_2) of the sol, which is a measure of oxidizable substrate, is only $\frac{1}{3}$ the amount in question. Further, when the extra oxygen consumption following one succinate addition is ended, and any substrate depending on it as a carrier fully oxidized, a second succinate addition again leads to greater oxygen use than theoretical. Malonate, which inhibits the succinic dehydrogenase, has no effect on the residual oxygen consumption of the solution, and finally, the total amount and rate of oxygen consumption when succinate and lactate are simultaneously or successively added is no greater than their separate sum.[3] In this system, therefore, there is no evidence for a carrier rôle of succinate. (Compare Greville, 1936.) The additional oxygen usage presumably serves to carry oxidations past the fumarate stage.

Because of these non-stochiometric relations attempts to analyze velocities and orders of reaction have had little success. The initial oxidation rate is independent of the concentration of succinate added over a considerable range. Yet the rate rapidly falls with time even when a large amount of succinate is initially present. Here the quantity left after a period of oxidation is still greater than that originally added in other experiments and which gave a maximal initial rate. An entirely analogous situation was encountered in the oxidation of lactate by *Sarcina lutea* (Gerard, 1930). Possibly substrate is relatively rapidly modified by one reaction to an intermediate form from which it is supplied to the catalytic system for oxidation at a rate dependent on the concentration of the intermediate.

SUMMARY

Rabbit brain was cytolyzed by grinding and suspending in 10 volumes of water and extracts made as follows: 1, the supernatant solution after centrifugation; 2, the washed centrifuge residue; 3, a preparation made from the sol by isoelectric precipitation, centrifuging and redissolving in dilute alkali. The oxidizing systems of these extracts were studied.

[3] In 6 experiments on rat brain sol, paralleled by 2 on rabbit, the initial rate in sol alone was 18 during a half hour. With nothing tipped, the rates for the following 10 minutes and one hour were 14 and 11; with succinate tipped, 35 and 22; with lactate tipped, 24 and 12; and with both, 56 and 25. The increase with both substrates for the hour is equal to the sum of the increases produced by each alone: (25–11) compared to [(22–11) + (12–11)]. For the first 10 minutes the total rate together is the sum of the separate rates, but the increase above sol alone is greater in the mixture. A slight carrier action may therefore be present.

All three respire to a small extent in the absence of substrate, the oxygen consumption of the sol being about one-tenth that of the brain from which it was prepared. Addition of the centrifuge residue to the sol produces a coenzyme-like effect on residual respiration, the total oxygen consumption of the two together being 20 per cent greater than the sum of their separate ones. The factor in the residue is heat stable, and some part of the system is inactivated by urethane.

The sol oxidizes methyl glyoxal, glycerophosphate, lactate, fructose and, with great vigor, succinate and paraphenylenediamine. The activity of the succinate oxidizing system is 2 to 3 times as great as that of intact brain. Szent-Gyorgyi's suggested carrier rôle for the succinate-fumarate system is not confirmed on this extract.

The glucose oxidizing system is destroyed (or inactivated) by cytolysis. The lactate system is not completely destroyed, but its components are separated by centrifuging, so that lactate oxidation by sol or residue alone is less than by the mixture.

M/1000 cyanide stimulates and M/100 cyanide inhibits the residual respiration of the sol. It also stimulates the oxidation of methyl glyoxal, partly itself exerting a catalytic action and partly increasing the action of the enzyme. Cyanide inhibits the oxidation of succinate and paraphenylenediamine by the sol, and methylene blue partially removes the inhibition of succinate but not of paraphenylenediamine.

The extract prepared by isoelectric precipitation has at least two enzyme systems still present, those involved in succinate and paraphenylenediamine oxidation. This extract, per unit dry weight, contains 6 to 8 times as much succinate oxidizing activity as the original brain, indicating a partial purification of the enzyme.

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THE ESTABLISHMENT OF THE DIURNAL TEMPERATURE CYCLE

N. KLEITMAN, S. TITELBAUM AND H. HOFFMANN[1]

From the Department of Physiology of the University of Chicago

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That the diurnal cycle of sleep and wakefulness is an individually acquired phenomenon depending upon the cerebral cortex for its establishment and maintenance was concluded from observations on decorticated dogs made by Kleitman and Camille (6). In attempting to elucidate the mechanism responsible for the development and persistence of this cycle, we found that it runs parallel with the diurnal body temperature curve (8) and diurnal variations in steadiness and performance of muscular and mental tasks (7, 9). The present report deals with the time of establishment of the diurnal cycle, as judged by the appearance of a definite diurnal body temperature curve, in the human subject.

Since the diurnal alternation of sleep and wakefulness is absent at birth, one would expect to find no diurnal variation in body temperature in the very young infant. However, data available in the literature show that even the new-born exhibit such a daily variation in temperature, sometimes amounting to over one-half a degree Centigrade. The earlier observers, such as Baerensprung (1), obtained fragmentary and unrelated figures, constructing composite curves based on temperature readings made at different hours on successive days, emphasizing morning and evening temperature levels, or contrasting sleep with wakefulness. The first systematic study was made by Jundell (5), who in a 100-page paper reported temperature observations, over periods varying from 2 to 11 days, on 47 new-born babies, some 50 infants between the ages of three weeks and nine months, three older children, and five adults. Jundell found that diurnal temperature range increased with age and he constructed composite curves for different age groups. Although he took body temperature readings six times daily, his composite curves are based on 12 or 18 points obtained by staggering the hours of observation for different subjects. Jundell's tables do not show the room temperature, even though Baerensprung's figures indicate some influence of the room temperature on the body temperature, and room temperature is usually higher in the daytime than at night.

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Our own observations were made on new-born babies at the Chicago Lying-In Hospital and on older babies and children at the Bobs Roberts Memorial Hospital. The new-born were entirely normal, and the older ones were in the hospital for observation or for the correction of nutritional disorders, and, with some few exceptions, were not "sick" and did not have fever. Their rectal temperature was determined every two hours, except that between midnight and 8 a.m. only one reading was made, at 4 a.m. The immediate environmental temperature was read off from a thermometer attached to the child's bed. The periods of sleep and wakefulness, of eating, and of activity were also noted. (For the purpose of comparison,

TABLE 1

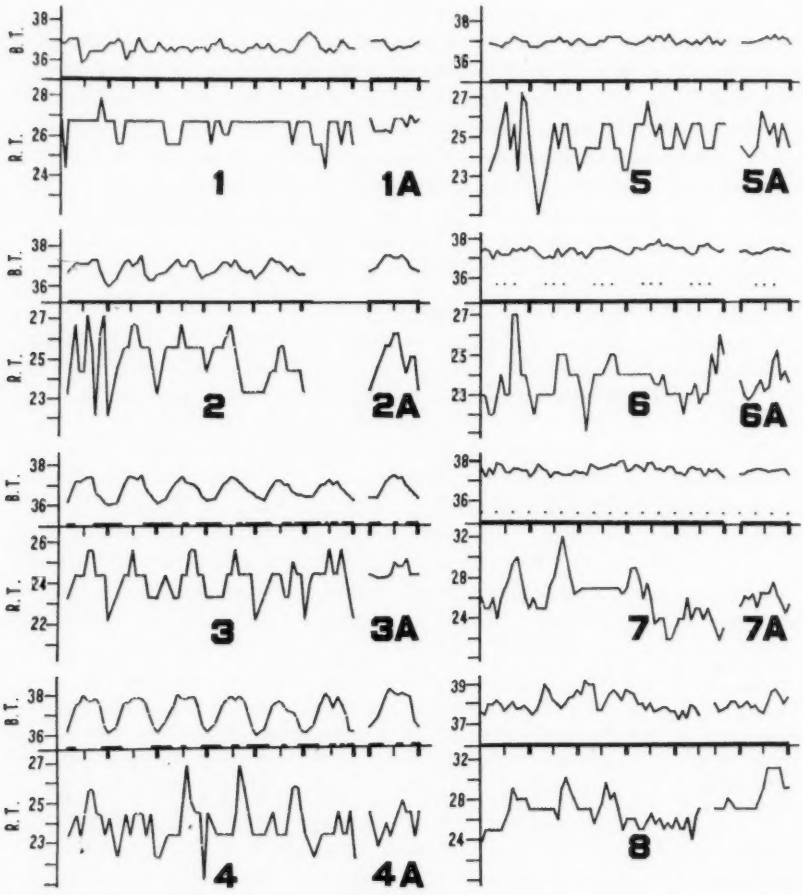
The ratio of the average diurnal temperature range in a given age group (AR) to the range of the average, or composite, diurnal body temperature curve for that group (RA)

Upper sets of figures based on our data; lower, on those of Jundell

AGE GROUP	NUMBER OF SUBJECTS	VARIATION IN INDIVIDUAL DIURNAL TEMPERATURE RANGES	GROUP AVERAGE OF THE DIURNAL TEMPERATURE RANGE (AR)	THE AVERAGE DIURNAL TEMPERATURE CURVE FOR THE AGE GROUP			$\frac{AR}{RA}$
				High	Low	Range (RA)	
		°C.		°C.	°C.	°C.	
New-born	10	0.35–0.74	0.62	36.95	36.68	0.27	2.30
1–5 mo.	5	0.62–1.02	0.80	37.28	36.72	0.56	1.43
7–11 mo.	6	0.74–1.15	0.98	37.38	36.56	0.82	1.20
14–22 mo.	6	1.18–1.80	1.67	37.51	35.95	1.56	1.07
3–6 yr.	4	1.39–1.86	1.56	37.50	36.15	1.35	1.16
7–13 yr.	4	1.10–1.82	1.44	37.73	36.36	1.37	1.05
New-born	18	0.17–0.53	0.38	36.86	36.76	0.10	3.80
New-born	15	0.23–0.63	0.44	36.87	36.72	0.15	2.93
New-born	14	0.17–0.67	0.39	36.96	36.70	0.26	1.50
<6 mo.	54	0.32–1.50	0.69	37.33	36.81	0.52	1.33
2–5 yr.	3	0.94–1.43	1.22	37.47	36.37	1.10	1.11
19–22 yr.	5	0.85–1.23	1.06	37.24	36.24	1.00	1.06

nearly 100 hospital records of non-fever patients of all ages (from a few weeks to 80 years) and comprising periods from 4 to 60 days were studied for the diurnal variations in temperature.)

From four to ten children of each of the following age groups were observed for about ten days: new-born, older ones up to 6 months, 7 to 12 months, 13 to 24 months, 25 months to 6 years, and 7 to 13 years. For each subject we determined the average daily temperature range (AR) and then the range of the average curve for the period (RA). We also noted the variations in the individual average diurnal temperature ranges, and constructed composite average curves for the different age groups. The room temperature data were handled in the same manner as



In all figures the body temperatures (B.T.) are plotted above and the room temperatures (R.T.) below the baselines. The temperatures are expressed in degrees Centigrade, and the scale is the same in all figures, except for room temperatures in figs. 7, 7A, and 8, where it is half that scale. The time is indicated in 12-hourly periods on the baseline, a thick mark indicating midnight, and a thin one, noon. In figures 3, 3A, 4, and 4A the thin portion of the baseline refers to periods of wakefulness. In figures 5, 5A, 6, and 6A the dots above the baseline indicate times of feeding. Figure 1A is plotted from the averages of the data used for figure 1, and the same applies to the other "A" figures.

Figs. 1 and 1A. Normal new-born baby, first 6 days of life.

Figs. 2 and 2A. Normal baby, 3½ months old. A suggestion of a regularity in the diurnal temperature curves.

Figs. 3 and 3A. Normal child, 15 months old. The ranges are about the same as in figure 2, but a much greater regularity and superimposability can be seen in

those for the body temperature. In addition, we looked for the relative constancy of the high and low temperature values as regards their magnitude and hours of occurrence. This constancy, or lack of it, could be determined for the individual or age group by obtaining the ratio of AR to RA, that ratio being high when the high and low temperature points were reached at different hours on different days, and approaching one as the diurnal temperature curves became more superimposable.

In table 1 is presented a summary of our temperature data, as well as Jundell's figures (5) arranged in a similar manner. It will be seen that there is a parallelism between these two sets of figures, but that our figures are uniformly higher than Jundell's. This may be due to the fact that our diurnal curves are based on ten observations daily, while his are based on only six. In a general way, the group average of the diurnal temperature range increases with age, but during the second year of life there appears rather abruptly a marked increase in the magnitude of that temperature range, which is now almost doubled in value and definitely exceeds that of older children and adults. This increased range involves a very low minimal temperature, as only in this age group does it fall below 36 degrees Centigrade. It will also be noted that the AR:RA ratios are high at first (also higher for our subjects than for Jundell's, and probably for the same reason) but come close to 1.00 before the children reach the age of two years, indicating a more complete diurnal regularity.

In figures 1 to 4A are shown the successive daily temperature curves as well as the average curves for a new-born baby, a baby 3½ months old, a 15-month old child, and one 9 years of age. We selected the 3½-months old baby as the one that had the highest diurnal temperature range in its age group, and the 15-month old child as the one with the lowest in that category. We thus obtained diurnal temperature ranges of about the same magnitude (1.02° and 1.18° respectively), although the average diurnal ranges for the two groups were 0.8° and 1.67°. Yet the successive daily temperature curves of the older child can be superimposed upon each other more easily than those of the younger (figs. 2 and 3).

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figure 3. During the first two days of observation the daytime naps were absent, and the temperature range for those days was greatest.

Figs. 4 and 4A. Normal child, 9 years old. Note the nicks in the daytime plateaus, coinciding with naps.

Figs. 5 and 5A. Child, 12 months old, with hydrocephalus.

Figs. 6 and 6A. Child, 9 years old, brain tumor, hydrocephalus, unconscious for one year. A suggestion of higher temperatures following the feedings. AR:RA = 1.53.

Figs. 7 and 7A. Same child, changed feeding routine: the three daily feedings separated by equal intervals. Suggestion of regularity in temperature curve (7A) is lost. AR:RA = 2.66.

Fig. 8. Same child, during two of the occasional periods of hyperthermia.

That living for a certain length of time in a world run on a 24-hour schedule is in itself not sufficient for the establishment of a pronounced diurnal temperature cycle can be seen from figures 5 and 5A, in which the body temperature variations of a 12-month old hydrocephalic child are plotted. No regular temperature variation is evident in figure 5, and the average diurnal range is only 0.46°, or in the category of the new-born. Fig. 5A shows a slight tendency for the room temperature to be higher in the daytime than at night, and the same is true of the average body temperature curve. Furthermore, the diurnal body temperature curve may be lost even after it had been presumably well established, as appears from figure 6, which represents the variations in body temperature of a child of 9 years that had developed a brain tumor and had been unconscious for about one year. Here, too, we find an average diurnal body temperature range of 0.58° only, with a suggestion of diurnal regularity (fig. 6A). That child had to be fed artificially, and was usually given three meals between 8 a.m. and 6 p.m. When the feedings were spaced equally, every 8 hours, the slight diurnal regularity was lost, as is shown in figures 7 and 7A, and the AR : RA ratio increased from 1.53 to 2.66, although the average daily temperature range remained unchanged (0.60°). Usually the temperature of this child fluctuated between 37° and 38°, but from time to time it would go through periods of hyperthermia with much greater temperature fluctuations. The temperatures for two such periods are plotted in figure 8, from which it can be seen that, with some delay and irregularity, the body temperature seems to vary with the room temperature.

From the hospital records we could get information only for the daytime temperature, from 8 a.m. to 8 p.m., but, in general, these data confirmed the results obtained directly, particularly as regards the gradual increase of diurnal temperature variation with age and the surpassing of the adult value during the second year of life.

Discussion. Our results demonstrate that while there may be a diurnal fluctuation in body temperature even in young infants, the diurnal temperature cycle, in the sense of a regularly recurring and superimposable variation, first appears during the second year of the child's life. A gradual approach to that state can be discerned during the first year in that the diurnal variation increases in magnitude practically from birth on. The factors responsible are partly exogenous and partly endogenous. Among the former, the diurnal variation in environmental temperature is of minor, if any, importance; the feeding routine which early in life is confined to the waking hours of the mother is probably of greater influence, as was shown by the effect of a change in the hours of feeding upon the body temperature of the nine-year old child with the brain tumor. The endogenous factor is probably the development of the nervous system

which permits of an adaptation of wakefulness and sleep to the alternating cycle of day and night. In passing from the polyphasic type of existence characteristic of the new-born to the monophasic seen in older children and adults, one of the first learned performances is sleeping through the whole night by skipping one feeding, and the gradual lengthening of the daytime periods of wakefulness. Instead of sleep being the prevalent mode of existence, with wakefulness interrupting it periodically for short intervals of time, we end up with the waking state comprising 15 to 17 hours out of the 24, and one continuous period of sleep, usually during the hours of darkness. As judged by the establishment of the body temperature curve, we would place the change in the second year of life because of the sudden, marked increase in the diurnal body temperature range and the approach of the AR:RA ratio to 1.00. Learning to walk, with its resulting widening of the sphere of the child's activity, is probably the outstanding influence during the second year of life. It is during this year that the average minimal temperature is below 36°C. It suggests that the almost incessant waking hour activity of children under two leads to greater fatigue and more complete muscular relaxation. Johansson (quoted from Jundell) demonstrated that even the adult can cut his diurnal temperature range in half by staying in bed, and we have shown (8) the marked effect of muscular tension and relaxation on the body temperature.

Unfortunately, earlier observers, like Baerensprung, Finlayson, Jundell, Gofferje, and Pilz, studied either babies under one or children of two years and over, and have missed the turning point, which occurs during the second year. But even Finlayson and Pilz reported a greater diurnal body temperature range in younger, as compared with older children and adults.

Another important feature, also noted by Finlayson, is the daytime temperature plateau with a rather sharp drop in the evening and an equally sharp rise in the early hours of the morning. In our figure 3, and more markedly in figure 4, it can be seen that the body temperature begins to drop in the evening long before the onset of sleep, and likewise begins to rise sharply shortly after midnight or long before awakening. If the sharp evening temperature fall is indicative of marked muscular relaxation, it would mean that the relaxation is the cause rather than the effect of sleep, and that it plays an important part in the mechanism of the diurnal cycle. We may be dealing with a vicious circle of wakefulness and sleep affecting the circulation and thus the metabolic activities of certain endocrine glands which, through the production of hormones, in turn affect the nervous system, fortifying the diurnal cycle of existence. That, in addition to the known factors which may be responsible for the establishment and maintenance of the diurnal temperature curve, there may be influences as yet unknown, appears from the mathematical analysis of several normal body temperature curves by Brouwer (2).

SUMMARY

1. During the first year of a child's life there is a gradual increase in the body temperature range and in the regularity of the diurnal temperature curve.

2. During the second year of life the adult type of diurnal temperature curve becomes definitely established, the range temporarily exceeding that of adult curves due to a lower minimal temperature.

3. The important endogenous factor is the adaptation of the child's routine of existence to the 24-hour cycle of alternating day and night, with respect to wakefulness and sleep, activity and rest.

We are indebted to P. Feiveson and H. Koch for their assistance in gathering the reported data; also to Drs. F. W. Schlutz, F. L. Adair, and P. Bucy for the facilities of their respective hospital services.

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FACTORS WHICH DETERMINE THE RATE AND DEPTH OF BREATHING[1,2]

ROBERT GESELL AND CARL MOYER

From the Department of Physiology, University of Michigan, Ann Arbor

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Scarcity of oxygen in the inspired air is known to produce two changes in breathing: accelerated respiratory rhythm and increased tidal air. Variations of this response and their significance seem, however, to have escaped attention. Sometimes the hyperpnea is limited to acceleration alone (fig. 1A), at other times it is limited to increased depth of breathing (fig. 5A). Between these two extremes there is every shade of variation (figs. 2A, 3A and 4A).

A comparison of the effects of intravenous injection of cyanide and of faradic stimulation of the saphenous nerve on pulmonary ventilation before and after vagotomy reveals differences of breathing indicative of the causes of the variations of anoxic hyperpnea (Gesell and Moyer, 1935). Before vagotomy the response runs importantly towards accelerated rhythm and after vagotomy toward increased depth of breathing which agrees with the findings of Scott (1908). From such observations we tentatively concluded that the vagi are essential for a high degree of acceleration; and that excitation of the respiratory chemoreceptors and of pain endings are essential to increased depth of breathing. More recent studies on vagal mechanisms controlling breathing indicate that the phenomena of rate and depth of breathing are inseparably combined (Brookhart, Steffensen and Gesell, 1936). It seems that any influence tending to increase the magnitude of inspiration can accelerate the rate of breathing through the participation of vagal function and conversely that any change in vagal function tending to alter the rate of breathing can modify the effect of the chemoceptive impulses and thus contribute to the control of depth of breathing. Accordingly, inherent differences in the pulmonary proprioceptive and respiratory chemoceptive reflexes should establish individualities in balance of the respiratory influences and account for the variability of response to anoxemia in the dog. With this as a working hypothesis we proceeded to record the respiratory response of anesthetized dogs (morphine-urethane) to oxygen scarcity (2.5 to 5 per cent O_2 in N_2) and

[1] Preliminary report—Proc. Soc. Exper. Biol. and Med. **32**: 849, 1935.

[2] This study was supported in part by a grant from the Rockefeller Foundation.

CO_2 excess (12 per cent CO_2 + 30 per cent O_2 in N_2) under varying respiratory denervations involving the proprio and chemoceptive paths.

RESULTS. We have done some twenty experiments of which five have been selected to represent the wide range of variations which occurs in anoxemia (figs. 1, 2, 3, 4, and 5). In no two of these experiments do the respiratory components appear to be similarly balanced under any variety

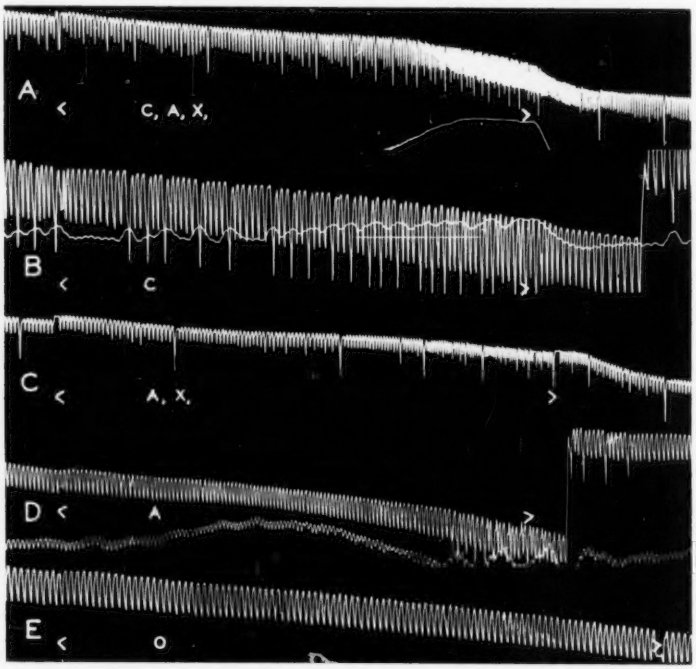

Fig. 1. Respiratory responses to standard anoxemia with varying types of sensory innervation involving the chemoceptors of the carotid and aortic bodies and the proprioceptives in the lungs. For a complete description see text. The letters in the figures indicate the innervations under consideration which are intact for any single observation. *A* = aortic chemoceptors, *C* = carotid chemoceptors, *X* = both left and right cervical vagi, *O* = complete *A*, *C* and *X* denervations. *LX* = left cervical vagus, *LPX* = left pulmonary vagal fibers.

of denervation. In no two are the effects of anoxemia under any state of innervation completely comparable. The results, therefore, offer an excellent opportunity of weighing the relative importance of the factors which we have studied.

Figure 2A shows an increase in rate and depth of breathing during anoxemia with innervation intact. This response resembled most closely the

pure acceleration type (fig. 1A), particularly near the close of the period of oxygen scarcity when the amplitude diminished below normal as the rate continued to increase. When next the checking mechanism of the inspiratory inhibitory fibers was removed by vagal block (2B) the previous increase in rate of breathing gave way to augmentation of the tidal air. This hyperpnea resembled the amplitude type of response of the intact animal to anoxemia (fig. 5A) in which acceleration is missing. Following this observation the vagus nerves were deblocked, the carotid gland regions denervated and anoxemia reëstablished. Breathing then became very rapid and shallow, changing to the pure acceleration type of response to anoxemia of the intact animal in figure 1A. After double vagotomy (2D), the stimulating effects of anoxemia on pulmonary ventilation were missing,

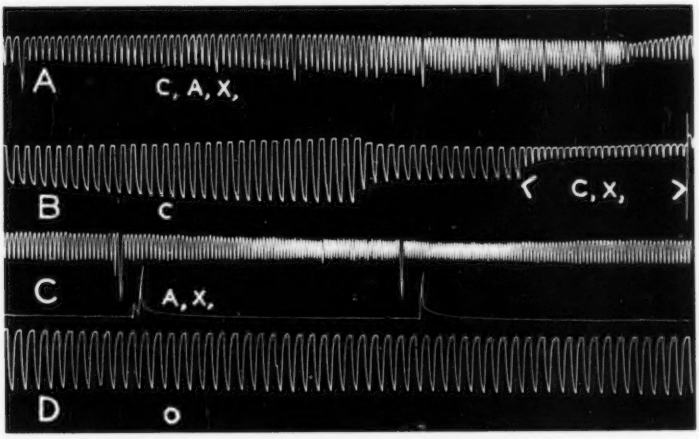

Fig. 2. Respiratory responses to anoxemia. For description see text and legend of figure 1.

which agrees with the findings of Heymans et al. and others. It may be concluded from this introductory experiment that modification of the proprio and chemoceptive innervation produces profound changes in the response to anoxemia and that such procedure offers a method of further analysis of the mechanisms controlling the rate and depth of breathing in the complete animal.

In animal 3, the first response to anoxemia, while innervation was still intact, exhibited a typical increase in rate and depth of breathing (fig. 3A). It differed slightly from 2A, in the greater increase of tidal air throughout anoxemia. The relatively high intensity of the amplitude component was further shown by the large increase in tidal air in observation 3B during anoxemia following vagal block. So far the findings in animals 2 and 3

differ only in degree but when the vagi were next deblocked and the carotid gland regions denervated (3C) the response to anoxemia was decidedly different from that of 2C. The augmentation of breathing was comparable to 3A with innervation intact but on a smaller scale. Before the following observation, 3D, the pulmonary branches of the vagus nerves were cut which left intact only the chemoceptive paths from the aortic bodies. In the absence of the vagal proprioceptive accelerating influence, the depth of breathing was now considerably increased, indicating that in this particular animal the amplitude influence arising in the aortic region as well as that

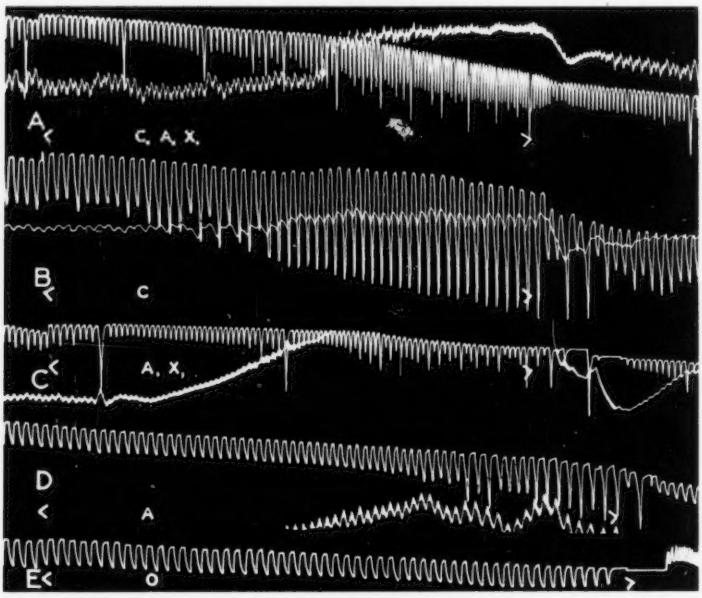

Fig. 3. Respiratory responses to anoxemia. For description see text and legend of figure 1.

arising from the carotid glands was strong (figs. 3B and 3D). After vagotomy, 3E, anoxemia had relatively little effect; only towards the close was there an obviously accelerated rate. Again we conclude that disturbances in balance of proprio and chemoceptive impulses importantly determine the respiratory response of an animal to anoxemia and that identical denervations in different animals are capable of producing different imbalance. It seems that this difference in imbalance depends on the inherent intensity or effectiveness of various factors contributing towards respiratory control.

A slightly modified set of procedures was used in animal four. The right vagus nerve was cut at once. This section presumably halves the influence arising from the pulmonary proprioceptive endings and from the aortic chemoceptors. Under these conditions (4A) anoxemia elicited a powerful response which slightly favored increase in amplitude over increase in respiratory rhythm. This relatively greater change in depth of breathing was probably due to section of the right vagal proprioceptive fibers and its consequent reduction of rate control. Left vagal block (4B) accentuated the disproportionate increase in depth of breathing still more. It will be seen that there was an absolute increase of amplitude and only a small increase in respiratory rhythm.[3] Next, 4C, the innervation was changed by deblocking the left vagus and denervating the carotid gland regions after which anoxemia evoked the acceleratory type of response with decreased tidal air similar to that noted in 2C. In 4D an entirely new procedure was tried. The aortic nerves were cut thus eliminating all known chemoceptive impulses. The left vagus was still deblocked, which retained the function of the left pulmonary vagal fibers. Anoxemia still accelerated breathing, even though no chemoceptors were available for stimulation. This striking response in the apparent absence of peripheral chemical excitation suggests a third important factor controlling respiration during anoxemia which will be considered later. After section of the pulmonary branches the previous effects of anoxemia were grossly changed. There was a slight increase in depth and rate of breathing followed by a decrease in depth and final respiratory paralysis.

The experimental procedures in animal 5 were identical to those in animal 4 yet the reactions to anoxemia were very different. The first anoxemia (5A) elicited a pure amplitude response, a type to be expected only after double vagotomy. Such results are indicative of ineffective mechanisms of rate control. Nevertheless left vagal block slowed the basal rate of breathing (5B) suggesting that those nerves were exercising some sort of rate control. With the proprioceptive and aortic chemoceptive impulses

[3] It should be noted that the basal rate of breathing was increased after right vagal block over that of (4A) where the left vagus nerve was still functioning. Similar acceleration from simultaneous double vagotomy is not uncommon and is probably due to the removal of a rate inhibiting effect of a continuous firing of the proprioceptive endings. Paradoxical as it may seem, such rate inhibiting influences were seen to be conducive under proper circumstances to a high degree of acceleration of respiratory rhythm (Brookhart, Steffensen and Gesell, 1935). They showed that the effect of any group of afferent impulses upon breathing is largely determined by other impulses simultaneously impinging on the central respiratory mechanism. An injection of cyanide which by itself only moderately accelerates the rate of breathing, when combined with a slowing effect produced by stretching of the lungs may provoke a rate of breathing far in excess of that from cyanide alone. A similar injection after vagotomy may actually retard. It is well to keep this paradoxical action of the vagus nerve in mind in the interpretation of the control of breathing.

now missing anoxemia produced the same increase in tidal air and the same lack of acceleration as in 4A which is suggestive of a relatively stronger influence of the carotid chemoceptors over that of the aortic chemoceptors on amplitude control. The carotid gland regions were now denervated and the left vagus nerve was deblocked (5C), which returned the pulmonary proprioceptive and aortic chemoceptive impulses. The increase

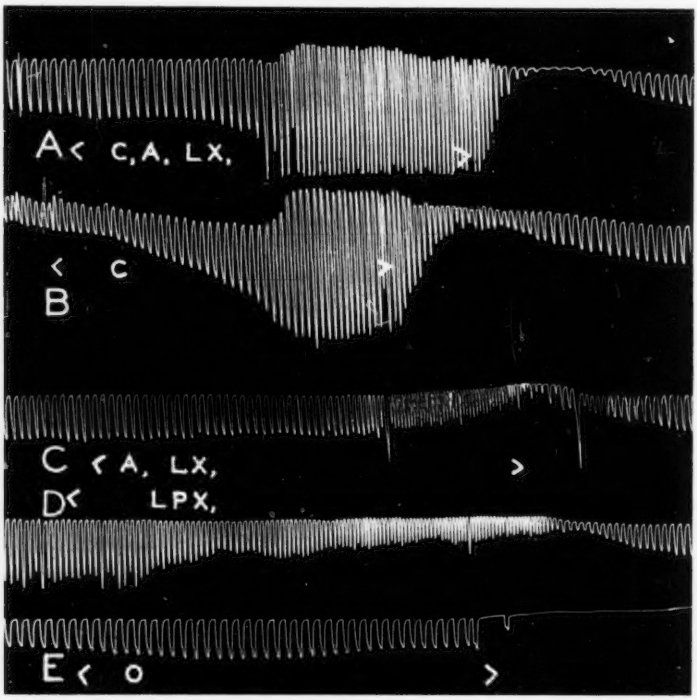

Fig. 4. Respiratory responses to anoxemia. For description see text and legend of figure 1.

in amplitude from anoxemia noted in 5A and 5B was thereby abolished and an increased rate of breathing elicited for the first time in this experiment. Further and complete removal of chemoceptive impulses by section of the aortic nerves (5D) were followed by even greater acceleration of breathing during oxygen scarcity. This compares with the behavior of animal 4. Final section of the left pulmonary fibers (5E) eliminated the acceleration noted in 5D. The main effect of anoxemia was depression.

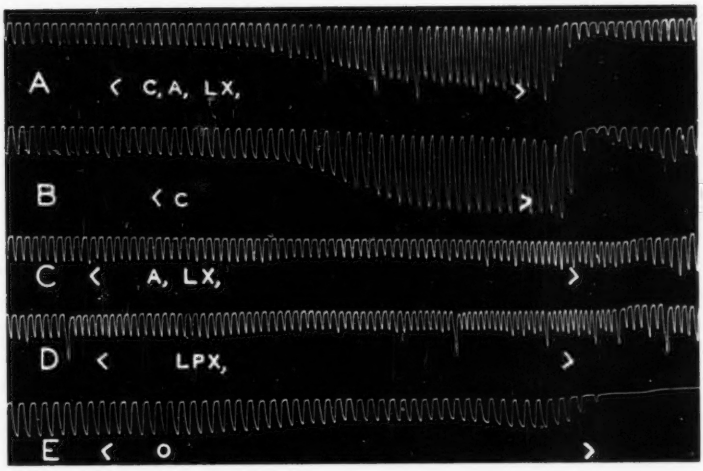

Fig. 5. Respiratory responses to anoxemia. For description see text and legend of figure 1.

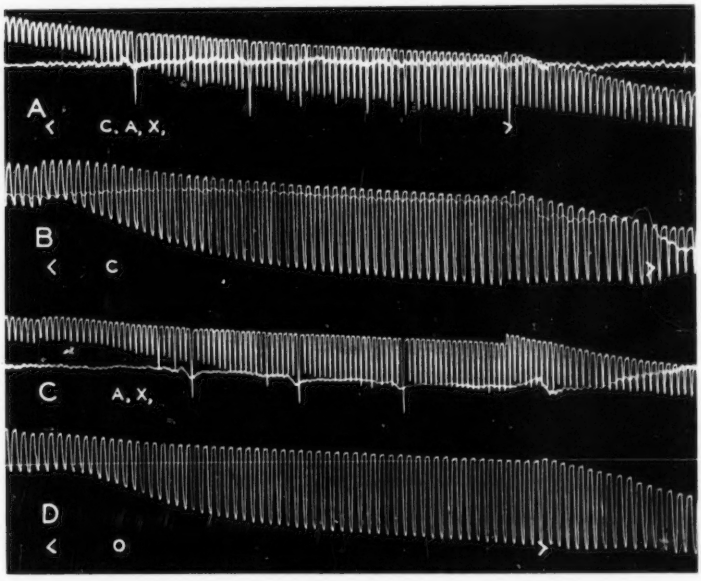

Fig. 6. Respiratory responses to hypercapnia. For description see text and legend of figure 1.

The response to anoxemia of animal 1 with innervation intact was diametrically opposite to that in 5A. The change in breathing was almost exclusively one of rate. After double vagal block (1B) the response to anoxemia was also radically different from that of (5B). There was very little augmentation of tidal air which is suggestive of a relatively small chemoceptive influence from the carotid glands. After deblocking of the vagi and denervation of the carotid gland region (1C) anoxemia produced acceleration but less than that in the intact animal. After section of the pulmonary vagal fibers (1D) leaving only the aortic nerves intact, anoxemia produced a small increase in depth of breathing and also some irregularity. Final section of the aortic fibers left ventilation unresponsive to anoxemia.

Until a complete explanation of the control of breathing is available unexpected phenomena are likely to arise. We have already alluded to one (see footnote 3) in which the addition of an inhibitory effect to an acceleratory effect augments this acceleration still more and in our present experiments we meet with other unexpected results in which removal of the influence of the chemoceptor impulses leads to as great or to even greater acceleration during anoxemia than before this denervation (4D and 5D). This latter paradox seems to be related to the central action of lack of oxygen. Since carotid gland denervation and vagotomy remove all known sources of peripheral chemical excitation and since such denervation leads to respiratory paralysis during anoxemia, Heymans (1931) and his associates concluded that lack of oxygen is without central excitatory action. When, however, the procedure of denervation of chemoceptors is conducted in another way, the results are very different. For example, anoxemia produced greater acceleration of breathing after a gross reduction of chemoceptors from denervation of the carotid glands (figs. 2C and 4C) than with innervation intact (figs. 2a and 4a). And after removal of all known chemoceptors by additional section of the aortic nerves, anoxemia still produced the same acceleration (fig. 4D). If all means of peripheral chemical excitation are thus removed how then was acceleration produced?

To be sure some unknown form of peripheral excitation in the lungs other than that at the proprioceptive endings is a possibility to be studied. On the other hand there is a logical and seemingly more pertinent criticism of the view of non-central excitation of anoxemia based as it is upon double vagotomy and carotid gland denervation experiments. The criticism is that such denervation is too inclusive. It removes not only the chemoceptive signals but another most important group of afferent respiratory signals, the pulmonary proprioceptive impulses. We, therefore, raise the question whether it is fair to test the central action of oxygen lack in the absence of the proprioceptive respiratory drive. Inasmuch as the central respiratory mechanism functions largely on incoming impulses, is such a

test not roughly comparable to testing a carburetor or the firing of a gasoline engine when the flow of gasoline is interrupted?

As to the nature of a central action of oxygen lack little can be said. An intensification of the vagal reflex which could bring about an earlier checking of inspiration and an earlier appearance of the succeeding expiration is conceivable and absence of a peripheral chemical drive as in (4D) would permit still earlier inhibition of inspiration and produce a shallow rapid type of breathing. The direct stimulation of breathing by localized cyanide poisoning of the medulla (Winder, Winder and Gesell, 1933) and the augmentation of simple spinal reflexes by local cyanide action on the spinal cord (Kaufman, 1936) are other instances illustrating the possible influence of impaired oxidation.

The respiratory response of the intact animal to the administration of carbon dioxide was on the whole more uniform than the response to oxygen lack. It consisted almost invariably of a substantial increase of tidal air with relatively little or even no acceleration of rate. Denervation of the chemoceptors before or after vagal block had no apparent effects on hypercapnic hyperpnea, for example the hyperpnea seen in 6a of the intact animal was almost a perfect duplicate of the hyperpnea in 6C after denervation of the carotid glands. Similarly the hyperpnea of 6B with the vagi blocked and carotid innervation intact was almost a perfect duplicate of the hyperpnea of 6D after double vagotomy and complete chemoceptor denervation. Vagotomy alone exerted definite effects noted primarily in the lower basal rate and the lack of acceleration during hypercapnic hyperpnea. The absence of effects of denervation of chemoceptors before and after vagotomy is in accord with the findings of Owen and Gesell (1931), Schmidt (1932), Selladurai and Wright (1932), Gemmell and Reeves (1933), Wright (1934), Wright (1936) indicating either a high or a definitely higher sensitivity of the central respiratory mechanism to CO_2 than of the peripheral chemoceptors.

Uniformity of response, preponderance of increased amplitude, minor acceleration and powerful central stimulation of breathing thus demark the response of CO_2 from the response to anoxemia. Evidence indicates that the lack of gross acceleration during hypercapnia with the pulmonary proprioceptors intact and chemoceptors denervated, as compared with the acceleration of anoxemia under similar conditions, is related not only to the augmenting action of anoxemia on the vagal reflex but to the depressing effects of CO_2 on the reflex as well (Gesell and Moyer, 1935). The central stimulating action of CO_2 which increases the intensity and duration of the discharge of the respiratory mechanism contributes further to the relative slowness of breathing.

In formulating a tentative and incomplete scheme of breathing as related to our experiments we have assumed a peripheral chemical drive for

anoxemia and a combined peripheral and central chemical drive for hypercapnia; a central augmenting action on the acceleratory reflexes (principally vagal) by anoxemia and a depressing action by hypercapnia. The stronger the chemical drive the stronger and more prolonged will be the central discharge unless it is in some way interrupted. The main source of interruption seems to be the vagal proprioceptive signals which increase with increasing depth of breathing. Increasing chemical drive, therefore, sets two processes into operation: an increasing inspiratory discharge and an accompanying increasing inspiratory inhibiting action of the vagi. Where increased inspiratory discharge keeps the upper hand increased amplitude is assured. Increased velocity of both phases of breathing permits the acceleration. If the accelerating action of the vagi is impaired, depth of breathing may predominate and acceleration suffer. Theoretically hypercapnia should produce such an effect through a process of chemical vagotomy (Gesell and Moyer, 1935). If the accelerating action of the vagi is markedly increased, through central action of lack of oxygen as it often seems to be, acceleration of breathing will predominate and amplitude will suffer (figs. 4C, 4D, 1C and 2C).

SUMMARY AND CONCLUSIONS

Oxygen scarcity in the inspired air usually increased both depth and rate of breathing. Sometimes the change in breathing was confined to increased depth and sometimes to increased rate.

The factors controlling these differences in depth and rate of breathing were studied by noting the effects of several types of denervations involving the chemoceptors in the carotid and aortic regions and the proprioceptive endings in the lung during anoxemia and hypercapnia.

Double vagal block, which left only the chemoceptors of the carotid glands intact favored increased tidal air at the expense of acceleration during anoxic hyperpnea. Section of the pulmonary vagal fibers after denervation of the carotid glands, leaving only the aortic region active gave similar results. Such response is due to reflex augmentation of the discharge of the respiratory center in the absence of the inspiratory inhibiting action of the vagi.

Denervation of the carotid gland regions which left the pulmonary proprioceptive and aortic body chemoceptive endings intact favored acceleration at the expense of decreased tidal air.

Complete denervation of peripheral chemoceptors (carotid and aortic) which left the pulmonary branches intact favored acceleration as much or even more. Such hyperpnea is suggestive of a central action of oxygen-lack facilitating the acceleratory reflex of the vagal proprioceptive fibers.

After complete denervation of chemoceptors plus vagal block anoxic hyperpnea was missing. Such failure of hyperpnea need not indicate

the absence of central effects of oxygen-lack. It can be theoretically attributed to the absence of the vagal proprioceptive drive and the susceptibility of its acceleratory action to modification through chemical changes occurring in the centers.

Hypercapnia produced a more standard type of hyperpnea than anoxemia. This held for all types of denervations. Preponderance of increased amplitude was characteristic. Denervation of the chemoceptors before or after vagal block produced no noticeable change in the hypercapnic hyperpnea which indicates that carbon dioxide exerts a stronger or more predominant stimulating action at the center than at the periphery.

It is tentatively proposed that rate and depth of breathing are importantly determined by the balance of rate and depth promoting reflexes and by central chemical control.

Vagal proprioceptive reflexes abbreviate the phases of the respiratory act and thus tend to assure a relatively rapid and shallow type of breathing as compared with that of vagotomy.

Chemoceptive reflexes (anoxemia and hypercapnia) and central stimulation (hypercapnia) augment the central respiratory discharge and in the absence of vagal acceleratory effects tend to produce a deep and slow type of breathing.

When both depth and rate promoting influences are strong a balance may be struck assuring a deep and rapid type of breathing for increased force of breathing automatically provides an increased stretching of the inspiratory inhibitory endings which prevents the slow apneustic type of breathing. Increased velocity of the two phases of breathing permit the acceleration. This type of breathing occurs in most animals during anoxemia and in some during hypercapnia.

When chemoceptor stimulation is relatively weak and the central vagal acceleratory mechanism is relatively strong conditions are favorable for an early checking of inspiration and a rapid shallow type of breathing such as is seen in the accelerated group in anoxemia.

When chemoceptor stimulation is relatively strong and the central vagal acceleratory mechanism is relatively weak conditions are favorable for a late checking of inspiration and a deep or slow type of breathing such as is seen in the retarded group in anoxemia.

When chemoceptor and central stimulation are strong and the central vagal accelerating mechanism is impaired conditions are also favorable for a late checking of inspiration and a deep slow type of breathing commonly seen in hypercapnia.

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THE EFFECT OF THYROTROPIC PITUITARY HORMONE ON THE OXYGEN CONSUMPTION OF THYROID TISSUE IN VITRO

R. K. ANDERSON AND HOWARD L. ALT

From the Department of Biochemistry, Tulane University of Louisiana School of Medicine, and the Departments of Chemistry and Medicine, Northwestern University Medical School

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Eitel, Krebs and Loeser (1) showed that thyroid tissue slices undergo hyperplasia when incubated in serum for 24 hours in the presence of thyrotropic pituitary hormone. In similar experiments with the thyrotropic hormone, Paal (2) observed a marked increase in the oxygen consumption of thyroid tissue. He recorded no measurements before the end of the 24-hour incubation period. While attempting to repeat Paal's work, we found that an increase in the oxygen consumption of thyroid tissue occurs almost immediately upon the addition of thyrotropic hormone.

The oxygen consumption determinations were performed in the Warburg apparatus (3) by the direct method, using oxygen in the gas space, 2 cc. homologous serum in the conical vessel and 0.2 cc. 10 per cent KOH in the well.[1] The thyroid tissue was obtained from dogs anesthetized with pentobarbital while kidney and liver tissues used for controls were obtained from similarly prepared dogs or from rats killed by a blow on the neck. Tissue slices varying from 15 to 50 mgm., fresh weight, were placed in each vessel. In each experiment, seven vessels with attached manometers were used, including one for a thermobarometer, three containing the tissue to be studied and three with the same tissue plus 0.2 cc. (10 guinea pig units) thyrotropic hormone.[2] (This relatively large amount of hormone was

[1] Although an increase in pH occurs in serum exposed to alkali, Gerard and McIntyre (4) have found that such a medium gives results similar to a control serum with added buffer or bicarbonate. We have observed a slightly higher oxygen consumption of rat liver tissue in non-buffered serum than in phosphate-Ringer solution.

[2] Dr. Paul Starr furnished the thyrotropic hormone, which was prepared for experimental purposes by Parke, Davis & Co., and is called antuitrin-T. It is said to be an aqueous solution of bovine anterior pituitary glands which contains the thyrotropic principle in purified form. It is substantially free from growth and gonadotropic hormones. It is non-irritating, is close to neutral in reaction, contains less than 1 per cent solids, and has a potency of about 50 guinea pig units per cubic centimeter according to present methods of assay.

used in order to obtain maximum effects on the thyroid gland.) About 20 to 30 minutes elapsed from the time the organs were removed until the tissue slices were shaking in the water bath at 37.5°C. After the system reached an equilibrium (approximately 20 minutes), manometric readings were made at 10 to 20 minute intervals. At the end of each experiment

TABLE 1

Effect of thyrotropic hormone on the oxygen consumption of dog thyroid tissue in homologous serum

DOG	TIME	Q_{O_2}*	Q_{O_2}* WITH THYROTROPIC HORMONE	PER CENT INCREASE
		Average of 3 determinations		
	hours			
1	1	5.5	8.1	47
	2	4.3	7.1	65
	3	4.3	6.4	49
	4	4.1	6.5	59
	5	3.0	4.2	40
	6	3.5	4.8	37
	7	3.2	4.4	38
2	1	3.2	4.9	53
	2	2.0	4.4	120
	3	2.0	4.1	105
	4	2.1	3.9	86
3†	1	3.0	3.5	17
	2	2.9	3.4	17
	3	2.0	2.7	35
4‡	1	3.0	3.8	27
	2	2.4	3.4	42
	3	2.1	2.9	38
5	1	5.0	6.1	22
	2	3.8	4.6	21
	3	3.4	5.0	47

* Q_{O_2} = cubic millimeters of O_2 consumed per milligram of dried tissue per hour.

† Thyroid gland obtained from dog previously treated with adrenal cortical extract.

‡ Thyroid gland about twice average size.

the tissues were dried and weighed, and the oxygen consumption calculated for several hourly periods. The figures listed in the table represent averages of three simultaneous determinations.

In the control experiments, it was found that thyrotropic hormone had no effect on the oxygen consumption of liver and kidney tissues of dogs

and rats. When the thyrotropic hormone was added to dog thyroid tissue, a distinct increase in the oxygen consumption occurred (see table). The increase was apparent during the first hour (17 to 53 per cent) and became more marked during the second or third hour. The variations in the control Q_{O_2} of the glands from different dogs may be attributed to the fact that the animals were recently-procured mongrels of varying age, sex and breed.

The mechanism of the increase in oxygen consumption of thyroid tissue in the presence of thyrotropic hormone is uncertain. Eitel and Loeser (5) stated that cellular changes in the thyroid glands of guinea pigs can be seen as early as two hours after the intraperitoneal injection of thyrotropic hormone. Thus, an early increase in oxygen consumption might be related to hyperplasia of the thyroid tissue. It is also possible that thyrotropic hormone might alter the metabolism of thyroid cells independently of any histologic changes.

These experiments support the belief that the thyrotropic pituitary hormone has a direct and specific effect on the cells of the thyroid gland. A similar type of study might prove useful in the investigation of other hormones.

CONCLUSION

Thyrotropic pituitary hormone causes an early increase in the oxygen consumption of thyroid tissue in vitro.

We wish to express our appreciation to Doctor Starr for his helpful suggestions during this work.

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A STUDY OF HEMOLYSIS IN VIVO BY THE APPLICATION OF THE BENZIDINE MICRO-COLORIMETRIC METHOD TO THE DETERMINATION OF FREE HEMOGLOBIN IN PLASMA[1]

DANIEL MELNICK AND GEORGE R. COWGILL

From the Laboratory of Physiological Chemistry, Yale University, New Haven, Connecticut

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In the course of an investigation on the influence of diet upon the regeneration of serum protein (Melnick, Cowgill and Burack, 1936) it was found, as was reported by others (Holman, Mahoney and Whipple, 1934; McNaught, Scott, Woods and Whipple, 1936), that with prolonged plasmapheresis the hematocrit values tend to fall to anemia levels unless red blood cells from donors are injected periodically. Although dogs are generally compatible with each other as regards blood transfusion and this is evidenced by successful initial injections of cells, subsequent transfusions may create a sensitization which renders the donors incompatible thereafter (Melnick, Burack and Cowgill, 1936). The development of immune isoagglutinins and isohemolysins results in the production of an intense hemoglobinuria; the continuation of the cell infusions, spaced from 7 to 10 days apart, is followed by symptoms of profound shock. The present communication is a report of a study of hemolysis *in vivo* subsequent to plasmapheresis and presents data concerning the normal extent of hemolysis subsequent to a single plasmapheresis and that resulting after the second cell infusion into the same dog from the same donor. This great increase in red cell destruction is one of the first signs of a developing incompatibility.

Preliminary plasmapheresis had indicated that normally the extent of hemolysis *in vivo* was small, in fact, hardly discernible by the naked eye. Therefore a method whereby small amounts of hemoglobin may be determined accurately had to be employed. In fact, many estimations had to be carried out using 1.0 cc. of the undiluted citrated plasma. Not all standard hemoglobin methods are sufficiently sensitive for this purpose. In the present study Wu's (1922) ultra-micromethod for the

[1] These data form part of a dissertation presented by Daniel Melnick to the Graduate School, Yale University, for the degree of Doctor of Philosophy, June, 1936. The expenses of this investigation were defrayed by a grant from the Research Fund, Yale University School of Medicine.

determination of hemoglobin was adapted as modified by Bing and Baker (1931) and McFarlane and McKenzie Hamilton (1932). This method is dependent upon the pseudo-peroxidase activity of hemoglobin.

Bing and Baker (1931) reported excellent agreement when the results obtained by the colorimetric technique were compared with the values calculated from oxygen capacity. In the present study the hemoglobin content of the standard solution was determined in triplicate by the Newcomer (1923) acid-hematin method. The disc factor[2] in the latter method had been carefully standardized by the Sendroy (1931) oxygen and the Van Slyke and Hiller (1928) carbon monoxide capacity methods, 12 independent observations having been made. The average of 3 determinations on an unknown blood sample by the benzidine colorimetric method was found to agree to within 0.3 per cent of that recorded in triplicate estimations employing the acid-hematin procedure. However, these determinations were conducted on normal dog's blood diluted according to the requirements of the respective methods. Studies of the benzidine micro-colorimetric method with respect to reproducibility of results, recoveries of added hemoglobin and sensitivity to slight variations in the hemoglobin concentration have indicated that this procedure, as adapted to the present study, is indeed very satisfactory for a study of hemolysis *in vivo*.

EXPERIMENTAL PART. *Description of the method.* Approximately 1.2 cc. of blood is drawn by means of a 5 cc. syringe from the jugular vein; 1.0 cc. is injected gently into a 3 cc. test tube (6 mm. diameter) accurately calibrated at the 1.0 and 2.0 cc. marks. The tube contains 1.0 cc. of 0.6 per cent sodium citrate in a 0.90 per cent sodium chloride solution. The anticoagulant and blood are mixed by inverting gently five times. The tube is then centrifuged at 2500 R.P.M. for 20 minutes and an aliquot of the *supernatant* fluid is used for analysis. One cubic centimeter of the hemoglobin solution is added to 2.0 cc. of a 2.0 per cent benzidine solution in 20 per cent acetic acid. The subsequent addition of 1.0 cc. of a 1.5 per cent hydrogen peroxide solution results in the production of a blue color which after 45 to 60 minutes is converted to a permanent red brown color of maximum depth. The reaction mixture is diluted to 25.0 cc. with 20 per cent acetic acid and compared with a standard prepared in similar fashion using 1.0 cc. of a blood sample containing 0.05 mgm. of hemoglobin per cubic centimeter.

The benzidine was carefully purified according to the directions given by Bing (1932). The hydrogen peroxide solution was prepared by diluting a superoxol solution that had been standardized against potassium permanganate.

[2] We are greatly indebted to Dr. James M. Orten for the use of this standardized disc.

The use of an equal volume of isotonic sodium oxalate solution, as the anticoagulant, proved to be unsatisfactory in that the oxalated plasma precipitated the benzidine from solution. The hematocrit (Van Allen, 1925) was determined simultaneously with each sampling.

In the adaptation of the benzidine micro-colorimetric method to a determination of free hemoglobin in plasma, a formula was developed, the derivation of which is given below.

Let

x = reading of the unknown,

and

s = reading of the standard,

then

$\frac{s}{x}$ = ratio of standard to unknown.

If

0.05 = milligram of hemoglobin per cubic centimeter of standard solution,

then

$\frac{0.05\,s}{x}$ = milligram of hemoglobin per cubic centimeter of unknown solution,

or

$\frac{0.5}{x}$ = milligram of hemoglobin per cubic centimeter of unknown solution when $s = 10$,

and

$\frac{50}{x}$ = milligrams per cent of hemoglobin in unknown solution.

If

p = cubic centimeter of plasma in the 1 cc. blood sample (1 − cell volume, as determined by the hematocrit)

and

a = cubic centimeter of the citrated plasma employed for analysis,

then

$\frac{p \cdot a}{p+1}$ = cubic centimeter of plasma in the aliquot taken for analysis,

and

$\frac{50}{x} \cdot \frac{p+1}{p \cdot a}$ = milligrams per cent of hemoglobin in the plasma.

Therefore,

$$\frac{50(p+1)p}{x \cdot p \cdot a} = \frac{50(p+1)}{x \cdot a} = \textit{milligrams per cent of laked hemoglobin in the blood.}$$

The formula actually employed is italicized. A typical example will illustrate the use and simplicity of the calculations.

With the hematocrit found to be 45 per cent, and 0.2 cc. of citrated plasma used in the analysis, x is found to be 10.1; then $\frac{50\ (1.55)}{10.1 \times 0.2} = 38.34$ mgm. per cent of laked hemoglobin in the blood.

The time required for the determinations may be shortened and the accuracy of the results increased by employing tubes, the upper portion of which measures 6 mm. x 60 mm., and the lower 60 mm. long with a smaller diameter such that it will hold 0.60 cc. With the tubes graduated for each 0.02 cc., the same blood sample and the same tube may be used for determining the hematocrit value as well as the concentration of laked hemoglobin.

Reproducibility of results. One cubic centimeter samples of citrated plasma were employed in these analyses. A slight amount of laked hemoglobin had been added to the citrated plasma. The results of four analyses indicated that independent determinatons upon the *same* sample of citrated plasma may be expected to agree to within ± 1.0 per cent of the average value.

Recoveries of added hemoglobin. The method was also tested by recovery experiments. Two samples of blood were drawn from 2 dogs, added to 3 per cent sodium citrate so as to make the final concentration of the anticoagulant 0.3 per cent. The citrated blood was shaken to create some hemolysis and then centrifuged. The citrated plasmas were then diluted, one portion with saline (1:1) and the other to the same extent with the standard hemoglobin solution (0.05 mgm./cc.). One cubic centimeter samples of the diluted plasmas were subjected to analyses. The results of seven determinations indicated that percentage errors in recoveries of added hemoglobin may vary from -6.8 to $+7.6$.

Sensitivity of the method. The benzidine colorimetric procedure for the determination of hemoglobin is a micro one and was originally intended for the determination of hemoglobin in micro samples of blood diluted from 2800 to 4000 times. Thus, it was found that the mere drawing of blood and the separation of the plasma from the cells resulted in some hemolysis. The degree of hemolysis in the normal plasma was then subtracted from the values recorded at the time of blood sampling during the study of *in vivo* hemolysis.

One cubic centimeter samples of blood, drawn from each of the jugular veins of four dogs, were added to the anticoagulant (citrate-saline) according to the procedure described above. One cubic centimeter aliquots of the citrated plasmas were used to determine the milligrams per cent of laked hemoglobin in the 1.0 cc. blood samples. The experimental findings are given in table 1.

Although the method is an accurate one for the determinations of laked hemoglobin in the same plasma sample, the agreements obtained between two blood samples, drawn simultaneously from the *normal* animal, were only within 9.33 per cent of the average value. However, the absolute values are *exceedingly small.*

The above experiment serves to emphasize the fact that the extreme

TABLE 1

Sensitivity of the method: The hemolytic effects of preparing normal blood for analysis ("manipulative" hemolysis)

DOG NO.	(HB.)* SAMPLE	(HB.) AVERAGE	DEVIATION FROM AVERAGE
	mgm. per cent	*mgm. per cent*	*per cent*
1	3.60 4.00	3.80	5.28
2	3.23 3.52	3.38	4.44
3	3.21 2.53	2.87	11.85
4	5.00 3.63	4.32	15.75
Average deviation			9.33

* The symbol, (Hb.), used in this table and in the subsequent table, represents the concentration of *free* hemoglobin liberated as a result of the laking of cells.

TABLE 2

Significance of "manipulative" hemolysis

EXPERIMENT (DOG NO.)	HOURS AFTER PLASMAPHERESIS	(HB.) SAMPLE	(HB.) AVERAGE	DEVIATION FROM AVERAGE
		mgm. per cent	*mgm. per cent*	*per cent*
IV (2)	$\frac{1}{6}$	9.9 10.7	10.3	3.9
	1	12.8 13.8	13.3	3.8
	3	19.8 19.3	19.55	1.3
	6	27.8 27.8	27.8	0.0
	$13\frac{1}{2}$	32.2 32.2	32.2	0.0
	$22\frac{1}{2}$	11.8 12.5	12.15	2.9
	98	4.6 5.6	5.1	9.8

sensitivity of the method makes it impossible technically to obtain perfect checks. Thus, in a study of *in vivo* hemolysis, that due to manipulative difficulties becomes significant only when the *in vivo* hemolysis is at a minimum. Consequently, in such conditions of minimal and negligible hemolysis the lack of agreement between duplicate determinations is at a maximum but is really insignificant because the absolute values are so small. However, when hemolysis *in vivo* is significant, the "manipulative" hemolysis becomes proportionally reduced, and there is a closer agreement between duplicate determinations. This hypothesis was confirmed experimentally; illustrative data are presented in table 2.

RESULTS. In order to evaluate the degree of *in vivo* hemolysis in terms of total milligrams of laked hemoglobin, the blood volumes of the experimental animals were first determined. The dye method of Hooper, Smith, Belt and Whipple (1920) was employed. Dog 1, a female wire-haired

TABLE 3

Preparation of cells for reinjection: Hemolytic effects

EXPERIMENT NO.	BLOOD FROM DONOR		HEMOGLOBIN FROM LAKED CELLS		
	Citrated blood	Cell volume	Citrated plasma	Saline washings	Locke's solution
	cc.	*cc.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
I	86	34	2.54	33.0	24.0
II	220	90	2.36	16.7	27.7
III	180	74	2.27	26.9	26.4
IV	165	66	7.20*	21.4	24.8

* The greater amount of laked hemoglobin in the citrated plasma obtained in experiment IV was expected, because the stirring rod became loose and scraped the sides of the vessel during the collection of the blood.

terrier, weighing 6.83 kilos had a blood volume of 606 cc. This value was the average of two determinations taken eight days apart (616 cc. and 595 cc.). Dog 2, a female mongrel, weighing 7.86 kilos had a blood volume of 575 cc. This value was the average of three determinations (567 cc., 596 cc. and 563 cc.). In the present study it was assumed that the blood volume does not change appreciably subsequent to a single plasmapheresis, since the volume of the injected cell suspension was equal to the total amount of blood withdrawn.

In each of the four experiments a common blood donor was employed, a large male collie. The blood collected from this animal was treated according to the standardized plasmapheresis technique described elsewhere (Melnick and Cowgill, 1936). Hemoglobin determinations were carried out on the plasma, saline washing and the Locke's solution in order to determine the hemolytic effects of the preliminary manipulations. From the results given in table 3, it appears that a uniform procedure for

TABLE 4

Hemolysis in vivo subsequent to plasmapheresis

DOG NO. (EXPER. NO.)	CELL EXCHANGE	TIME AFTER PLASMAPHERESIS	HEMOGLOBIN FROM LAKED CELLS	
			Concentration	Total
	cc.	*hours*	*mgm. per cent*	*mgm.*
1 (I; 1st plasmapheresis)	Bled, 30 Injected, 20	0	1.2	7.3
		1	6.0	36.4
		3	5.9	35.8
		5	5.6	34.0
		7	4.4	26.7
		10	3.4	20.6
		26	0.0	0.0
1 (II; 2nd plasmapheresis)	Bled, 65 Injected, 88	0	6.0	36.4
		$\frac{1}{6}$	26.5	160.5
		$1\frac{1}{2}$	97.2	588.0
		$4\frac{1}{2}$	187.2	1,134.0
		$9\frac{1}{2}$	140.2	850.0
		21	124.2	753.0
		28	115.2	698.0
		48	116.2	705.0
		96	5.2	31.5
		120	0.9	5.5
2 (III; 1st plasmapheresis)	Bled, 67 Injected, 66	0	3.6	20.7
		$\frac{1}{6}$	9.2	52.8
		$1\frac{1}{4}$	15.0	86.5
		$3\frac{1}{2}$	15.2	87.5
		$5\frac{1}{2}$	16.4	94.4
		$7\frac{1}{2}$	11.1	63.8
		$10\frac{1}{2}$	8.3	47.8
		$13\frac{1}{2}$	3.3	19.0
		$17\frac{1}{2}$	1.5	8.7
		$21\frac{1}{2}$	1.1	6.3
		$24\frac{1}{2}$	0.0	0.0
2 (IV; 2nd plasmapheresis)	Bled, 44 Injected, 60	0	3.0	17.2
		$\frac{1}{6}$	6.7	38.5
		1	9.6	55.1
		3	16.0	92.0
		6	24.2	139.2
		$10\frac{1}{2}$	51.9	298.0
		$13\frac{1}{2}$	28.6	165.0
		$22\frac{1}{2}$	8.5	48.8
		$28\frac{1}{2}$	13.1	75.5
		$48\frac{1}{2}$	12.0	69.2
		98	1.5	8.6

The figures for laked hemoglobin have been corrected by subtracting from the observed values that amount due to the hemolytic effects of taking and preparing the blood samples for analyses. "Manipulative" hemolysis was found to be 3.4 mgm. per cent in experiment I; 3.8 mgm. per cent in experiment II; and 3.6 mgm. per cent in experiments III and IV.

In experiment II a hemoglobinuria was noted, beginning within $4\frac{1}{2}$ to $9\frac{1}{2}$ hours after plasmapheresis and continuing through the 48th hour. The amount of hemoglobin lost in the urine was not determined.

preparing the cells for reinjection was employed. The greatest deviations observed in the saline washings were doubtless to be expected because the cells were stored as a suspension in physiological saline until needed for injection two days later. Referring to the initial bleeding phase, one might conclude that the particular 'manipulative" hemolysis associated with that procedure produces about 2.5 mgm. per cent hemoglobin (see column 4, table 3).

Plasmapheresis was performed on dogs 1 and 2 using in all experiments the same technique (Melnick and Cowgill, 1936). Immediately after the cell infusions, and at stated time intervals thereafter, blood samples were withdrawn and subjected to analyses. The results are listed in table 4.

Discussion. In all of the four experiments performed, the determination of hemoglobin on the citrated plasma, saline washing and the modified Locke's solution, in which the red cells were suspended for reinjection, indicated a remarkable uniformity in the hemolytic effects of these preliminary manipulations. The *initial* infusions into the dogs of cells from the same donor produced a *proportional* but *negligible* hemolysis. However, when cells from this donor were injected a *second* time approximately 2 weeks later, a marked hemolysis *in vivo* was observed which failed to be related proportionally to the volume of cells injected. Thus, in the case of dog 1 the maximal hemolysis, over and above a hemoglobinuria, was more than 30 times that recorded after the first cell infusion, although the cell volume injected was only $4\frac{1}{2}$ times greater. In the case of dog 2, where similar cell volumes were injected, the extent of hemolysis was found to be more than 3 times greater subsequent to the second transfusion. Furthermore, after the second cell infusions the hemolysis was still manifest 48 to 96 hours later.

The hypothesis has been advanced (Melnick, Burack and Cowgill, 1936) that the development of blood incompatibilities in dogs results from a sensitization induced in the recipient by repeated injections of an antigen in the red blood cells of the donor. Using as indicators the sensitized dogs described in the previous communication, it was shown that approximately 50 per cent of the dogs tested at random are in the same hematological group as the incompatible donors. Dogs 1 and 2, used in the present experiments, are of the same group as the sensitized recipients referred to in the previous paper. Thus, this picture of increased hemolysis *in vivo* is to be expected only when the dog used as the donor is not of the same group as the recipient. At present we are studying whether dogs of the incompatible donor group can be sensitized by injecting washed red blood cells obtained from animals of the same group as our hitherto sensitized recipients. The possibility exists that no incompatibility may develop as a result of this procedure.

In our attempts to evaluate dietary factors with respect to their ability to promote the regeneration of serum protein (Melnick, Cowgill and Burack, 1936) we have been constantly aware of the fact that a fraction of the injected erythrocytes is broken down and that the liberated hemoglobin is completely metabolized. We have tried to evaluate this variable in terms of increased dietary protein consumption by use of the benzidine colorimetric procedure as a means for estimating the amount of free hemoglobin in the plasma. However, such a procedure is of only limited value since the liberated globin may by its amino acids supplement any deficiencies characteristic of the dietary protein, and thus exert a supplementary as well as an additive effect.

It seems to us that the method, as outlined in the present paper, is admirably suited for the determinations of the renal threshold for hemoglobin excretion. Reports of values in terms of body weight have been frequently given (Manwell and Whipple, 1929; Lichty, Havill and Whipple, 1932). It is obvious that the expression of a renal threshold in units, such as milligrams per kilo of body weight, can only be semiquantitative because the blood volume and body weight do not necessarily parallel each other. By the benzidine micro-colorimetric method values may be reported, as in the case of other blood constituents, in terms of 100 cc. of blood or plasma.

Recently Sunderman and Austin (1936), in their studies of the measurement of serum volume, had occasion to use the benzidine test in order to determine the critical concentration of hemoglobin that might be present and still have no influence upon the colorimetric readings. The method employed was to determine the dilution of the serum which would fail to give a positive benzidine reaction. Such a procedure was found to be satisfactory for their purpose. However, a more precise measurement of the concentration of free hemoglobin in the serum is possible by the use of the method as outlined in the present paper.

SUMMARY

1. The benzidine micro-colorimetric method for the determination of hemoglobin has been adapted to a study of hemolysis *in vivo*. The validity of the procedure has been tested and found to be satisfactory.

2. By determining the concentrations of free hemoglobin in plasma, it was shown that one of the first signs of blood incompatibilities developing between dogs is marked hemolysis *in vivo*. Although the initial infusion of red blood cells results in a negligible laking of the erythrocytes, a subsequent injection approximately two weeks later is characterized by a marked hemolysis *in vivo* which fails to be related proportionally to the amount of cells injected.

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THE ANTICOAGULATIVE ACTION OF ORGANIC ACIDS, AND OF HEPARIN AND THE ORGANIC BASE, DIETHYLAMINE[1]

AUGUSTUS WADSWORTH, FRANK MALTANER AND ELIZABETH MALTANER

From the Division of Laboratories and Research, New York State Department of Health, Albany

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Studies of the chemical reactions of cephalin and ionized calcium as they relate to the coagulation of the blood have been recorded in a number of previous reports (1–5); in this one, attention is directed to the inhibitory action of anticoagulants in relation to the calcium and cephalin of the plasma.

The oxalates, citrates, and fluorides depress the ionization of calcium. The action of the complex substances heparin and hirudine is not so clearly established. Jorpes (6), also Schmitz and Fischer (7) in their reports on heparin, and Bergström (8) as well as Chargaff, Bancroft and Stanley-Brown (9) in their studies of sulfonated carbohydrates, have attributed the inhibitory action to the acidic groups; Kühnau and Morgenstern (10) and Sterner with Medes (11) suggest that the anticoagulative action of compounds like cysteine or glutathione is due to their sulfhydryl groups. Tricalcium phosphate and magnesium hydroxide, as previously reported (1, 5), act by virtue of their basic properties.

The present investigation deals with the action of heparin. In addition, simple organic acids were included because the chemical groups under consideration dominated their chemical behavior. Urea, possessing two amino groups but neutral in reaction, two of the ureides with acidic properties, and a comparatively strong organic base, diethylamine, were also studied.

In testing these substances, molecular equivalents were dissolved in physiological-salt solution and the action of appropriate dilutions tested by the technic previously employed for determining the clotting activity of titrated amounts of calcium chloride and cephalin with oxalated plasma and also with dioxalated plasma after preliminary incubation with serum (5). Both of these methods are precise and the results obtained with them

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are readily confirmed. The anticoagulative action of these substances alone and in combination with varying dilutions of hydrochloric acid and sodium hydroxide was then determined.

TECHNIC. *Oxalated plasma* was secured by bleeding rabbits with a paraffined glass cannula from the carotid into paraffined tubes containing sufficient 1.0 per cent sodium-oxalate solution to give a final concentration of 0.1 per cent; the cells were removed by centrifugalization as recorded in earlier papers. To 0.1 cc. quantities of the cell-free oxalated plasma, 0.1 cc. of the different dilutions of anticoagulants was added, then 0.1 cc. of 0.01 per cent cephalin and sufficient physiological-salt solution to give a total volume of 0.6 cc. were added. One-tenth cubic centimeter of N/50 calcium chloride was placed in each tube. The mixtures were incubated in the water bath at 37°C. and the clotting time was recorded. The anticoagulative unit was taken as the amount which inhibited clotting during twenty-four hours, whereas without anticoagulant clotting occurred in ten minutes or less.

Serum for use in the experiments with dioxalated plasma was obtained by adding 4 volumes of 0.035 per cent calcium chloride (made up in physiological-salt solution) to 1 volume of oxalated plasma and defibrinating the mixture with a glass rod at the inception of clotting. This serum was allowed to stand overnight in the cold room to allow it to reach equilibrium—indicated by its failure to clot dioxalated plasma alone.

Dioxalated plasma was prepared by diluting 1 volume of oxalated plasma with 4 volumes of 0.2 per cent sodium oxalate. In determining the anticoagulative action of the different substances tested, 0.1 cc. each of serum, of dilution of the anticoagulant, of cephalin, and of N/50 calcium chloride were mixed and the total volume made up to 0.6 cc. The mixture was incubated in the water bath at 37°C. for six minutes when 0.1 cc. of dioxalated plasma was added to each tube and the tests reincubated. The controls, without plasma, clotted in one minute. The anticoagulative unit was taken as the amount of anticoagulant which inhibited the clotting during twenty-four hours.

In determining the effect of sodium hydroxide and hydrochloric acid, 0.1 cc. quantities of dilutions of sodium hydroxide and hydrochloric acid varying in concentration from N/10 to N/320 were pipetted into a series of tubes. The anticoagulative dose of the various tested substances and the other reagents were then added and the tests with oxalated plasma and with serum and dioxalated plasma carried out as described above. Controls, without acid or alkali and without anticoagulant, were included with each test.

The results of the direct inhibition of the coagulation of oxalated plasma and of the coagulative action of serum for dioxalated plasma differed only in that the latter required approximately half the amount of anticoagulative substance to inhibit clotting completely (24 hours) under the conditions of testing. The results of the experiments with oxalated plasma are summarized in table 1 which records the chemical character and anticoagulative activity of a group of acidic substances comprising mono-, di-, and tricarboxyl aliphatic acids and of two aromatic monosulfonic acids, one containing in addition a carboxyl group. The anticoagulative action is directly proportional to the titratable acidity, irrespective of the chemical character of the groups involved or of the hydrogen-ion concentration.

In table 1 there are also given the results obtained with urea, diethylamine, and heparin, as well as those with mono- and dicarboxyl amino acids, with the hydrochloride of the sulfhydryl amino acid—cysteine—and with two heterocyclic derivatives of uric acid. The anticoagulative action of the latter group is directly proportional to the titratable acidity, irrespective of the chemical character of the acidic groups. The amino acids containing single amino and carboxyl groups do not react as anticoagulants on account of the neutralization of one group by the other. The anticoagulative properties of cysteine hydrochloride are due to hydrochloric acid liberated as the result of hydrolysis. Glycine, which has neither

TABLE 1

Chemical characteristics of organic acids, of urea, diethylamine, and heparin: their anticoagulative effect on equal volumes of oxalated plasma in the presence of limiting amounts of calcium chloride and cephalin

	DISSOCIATION CONSTANTS	CHEMICAL TITER	ANTICOAGULATIVE TITER
Lactic acid	1.38×10^{-4}	Monobasic	M/25
Oxalic acid	3.8×10^{-2}	Dibasic	M/50
Citric acid	8.2×10^{-4}	Tribasic	M/75
Succinic acid	6.6×10^{-5}	Dibasic	M/50
Pyruvic acid		Monobasic	M/25
Galacturonic acid		Monobasic	M/25
Sulfanilic acid	6.2×10^{-4}	Monobasic	M/25
Sulfosalicylic acid	4.0×10^{-1}	Dibasic	M/50
Glycine	1.8×10^{-10}	Neutral	None
Aspartic acid	1.5×10^{-4}	Monobasic	M/25
Glutamic acid	4.1×10^{-5}	Monobasic	M/25
Cysteine hydrochloride		Monobasic	M/25
Parabanic acid	7.5×10^{-7}	Monobasic	M/25
Alloxan	2.3×10^{-7}	Monobasic	M/25
Urea	1.5×10^{-14}	Neutral	None
Diethylamine	1.26×10^{-3}	Monoacidic	M/100 to M/50
Heparin		Weak base	0.01 per cent solution

sulfhydryl nor hydrochloride groups, has no anticoagulatve action until an equivalent amount of hydrochloric acid is added.

When varying concentrations of hydrochloric acid and sodium hydroxide were added to these mixtures, it was found that with the exception of oxalic acid the anticoagulative properties of all of these substances, including cysteine hydrochloride, were completely lost when equivalent amounts of sodium hydroxide were used. The sodium salt of oxalic acid possessed the same anticoagulative action as the acid equivalent of oxalic or other organic acids.

Urea is neutral and not anticoagulative. Diethylamine, on the other

hand, is twice as effective in inhibiting the coagulation of oxalated plasma by cephalin and calcium chloride as the organic acids, on the basis of molecular concentration. The heparin[2] used was about seven times more active than diethylamine, on a percentage basis.

Whereas the activity of the organic acids was neutralized by an equivalent of sodium hydroxide, that of diethylamine was neutralized by the chemical equivalent of hydrochloric acid. Similarly, the anticoagulative action of heparin is also completely neutralized by hydrochloric acid. In fact, quantities of either heparin or diethylamine which possess the same degree of anticoagulative action are rendered inactive by similar amounts of acid. Hence it appears that the active group responsible for the anticoagulative action of heparin is basic rather than acidic in character.

In the light of our knowledge of the interaction of calcium chloride, cephalin, and protein, further study was directed toward determining the effect of heparin on the reactions of cephalin with calcium chloride and with protein.

TECHNIC. The technic of the first series of experiments in this group was the same as that used in determining the anticoagulative activity of the organic acids with the exception that the dose of anticoagulant was constant and the amounts of cephalin and calcium chloride were varied.

In the second series of this group of experiments, the dose of calcium chloride used was maintained constant and was the amount determined to be maximum in the first series. The amounts of cephalin and of anticoagulant (heparin) were varied.

In the third series the amount of cephalin used was determined by preliminary titration. It was the amount of cephalin which in the presence of calcium chloride and an appropriate amount of rabbit serum previously inactivated at 56°C. for one-half hour caused complete precipitation of the cephalin and protein present. To this constant quantity of cephalin varying amounts of anticoagulant were added followed by constant amounts of inactivated serum and calcium chloride. The degree of precipitation was read after one hour. Oxalated plasma was then added. The mixtures were incubated at 37°C. and the clotting time recorded during twenty-four hours.

In the first series of experiments a constant dose of heparin, sufficient to inhibit the clotting activity for twenty-four hours, was added to serum or oxalated plasma; varying amounts of cephalin and calcium chloride were then added and the clotting time of the oxalated plasma or of added dioxalated plasma recorded. Maximum neutralization was not independent of the calcium concentration but required a definite quantity of both calcium chloride and cephalin.

In the next series a constant dose of calcium chloride was used and the amounts of heparin and cephalin varied. An experiment with the use of serum and dioxalated plasma illustrating the results is shown in table 2.

[2] The heparin used in this study was Lot no. 10-1 obtained from the Connaught Laboratories of the University of Toronto.

TABLE 2

The quantitative relation between cephalin and heparin as determined by the inhibiting effect of mixtures of these substances on the clotting activity of serum

CEPHALIN, 0.3 PER CENT	CLOTTING TIME OF 0.2 PER CENT HEPARIN DILUTED					
	1:80	1:160	1:320	1:640	1:1280	1:2560
Undiluted	0	0	0	24h	125m	34m
1:2	0	82m	53m	26m	18m	19m
1:4	82m	48m	24m	17m	12m	12m
1:8	24h	48m	32m	18m	12m	12m
1:16	24h	24h	87m	60m	33m	14m
1:32	24h	24h	24h	81m	28m	10m
1:64	24h	24h	24h	24h	86m	13m
1:128	24h	24h	24h	24h	24h	49m

TABLE 3

The parallel inhibition of the precipitation of cephalin protein and of the clotting of oxalated plasma, by heparin

REAGENTS	QUANTITIES						
	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Cephalin, 0.3 per cent	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Heparin, 0.5 per cent	0.4	0.2	0.1	0.1 (1:10)	0.1 (1:50)	0.1 (1:100)	0
Rabbit serum heated at 56°C. for ½ hr.; 1:100	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Physiological salt solution	0	0.2	0.3	0.3	0.3	0.3	0.4
Calcium chloride, N/10	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Precipitation	−	−	±	2+	3+	4+	4+
Oxalated rabbit plasma	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Clotting time during 24 hrs	No clot	No clot	No clot	Shreds of fibrin overnight	54m	28m	5m

TABLE 4

The parallel inhibition of the precipitation of cephalin protein and of the clotting of oxalated plasma, by diethylamine

REAGENTS	QUANTITIES					
	cc.	cc.	cc.	cc.	cc.	cc.
Cephalin, 0.3 per cent	0.1	0.1	0.1	0.1	0.1	0.1
Diethylamine	0.1 (1:10)	0.1 (1:50)	0.1 (1:100)	0.1 (1:200)	0.1 (1:400)	0
Rabbit serum heated at 56°C. for ½ hr.; 1:100	0.1	0.1	0.1	0.1	0.1	0.1
Physiological-salt solution	0.2	0.2	0.2	0.2	0.2	0.3
Calcium chloride, N/10	0.1	0.1	0.1	0.1	0.1	0.1
Precipitation	−	±	±	+	2+	4+
Oxalated rabbit plasma	0.1	0.1	0.1	0.1	0.1	0.1
Clotting time during 24 hrs	No clot	No clot	No clot	Clot overnight	41m	6m

In the presence of a constant quantity of calcium chloride the amounts of heparin and cephalin with which the same degree of coagulative activity was observed were directly proportional to each other.

In a third series of experiments varying quantities of heparin or of the base, diethylamine, were mixed with a constant dose of cephalin and an amount of inactivated serum added sufficient to precipitate completely with this dose of cephalin in the presence of an excess of calcium chloride. The mixtures were allowed to stand until precipitation in the controls was complete. Oxalated plasma was then added to the mixtures and the clotting time recorded.

The results recorded in table 3 demonstrate clearly that heparin prevents the precipitation of calcium-cephalin or cephalin-protein compounds and inhibits in similar degree the clotting of added oxalated plasma. Table 4 shows that diethylamine acts in the same manner as heparin.

SUMMARY AND CONCLUSIONS

Organic substances possessing acidic groups inhibit clotting to a degree determined by their titratable acidity. The hydrogen-ion concentration is of minor importance. The anticoagulative activity of the acidic substances was quite similar in degree to that of sodium oxalate and, as with this salt, is in all probability due to the depression of the calcium-ion concentration. Cysteine is without anticoagulative action; cysteine hydrochloride inhibits like other monobasic acids because of the acidic properties of the hydrochloride group it contains.

Heparin and the organic base, diethylamine, possess similar anticoagulative properties. Cephalin neutralizes the anticoagulative action of heparin in directly proportional amounts. Heparin reacts with the acidic group of cephalin, thereby preventing not only the interaction of cephalin and calcium chloride but, consequently, the formation of cephalin-protein complexes and the clotting of blood or plama. The inhibitive reactions of heparin and of diethylamine with cephalin are strictly parallel.

Since this paper was submitted for publication, the anticoagulative action of liquoid (polyanethosulfonic acid sodium) has been determined to be one-half that of the heparin used in these studies and its action similar in every respect to that of heparin or of diethylamine.

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THE INFLUENCE OF DIET UPON UREA CLEARANCE IN DOGS

R. C. HERRIN, ALLEN RABIN AND R. N. FEINSTEIN

From the Physiological Laboratory, University of Wisconsin, Madison

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Diet has been demonstrated to exert a marked influence upon the activity of the kidney in excreting urea. In 1923 Addis and Drury (1) reported that the excretion of urea relative to blood urea was increased in the human subject three to five hours after the ingestion of a mixed meal, glutamic acid or milk. Cane sugar or alcohol had no apparent effect in accelerating the excretion of urea. Jolliffe and Smith (2) (3) found that in the case of dogs, either fasting or on a low protein diet, the urea clearance was reduced, whereas a meat diet greatly increased both creatinine and urea clearances. Dietary effects of the meat were present sixteen to eighteen hours after the last meal. This effect of protein in the diet was also found by Van Slyke and associates (4) both before and after the kidneys were denervated by section of the nerves or novocainization of the renal pedicle. Pitts (5) found that meat, casein, glycine and alanine increased both xylose and urea clearances in dogs. Increasing endogenous protein metabolism with throxine or phlorizin had a similar effect.

This study is concerned with the possible effect of other dietary factors upon urea clearance in dogs. Meat and casein which had been precipitated seven times with HCl, were almost equally effective in raising both post-prandial and post-absorptive urea clearance. Glycine, alanine, glutamic acid and glycine synthesized from mono-chlor-acetic acid increased the post-prandial urea clearance. Inasmuch as the residue from the deaminization of glycine by nitrous acid was equally effective in raising urea clearance the study was extended to other carboxylic acids.

EXPERIMENTAL PROCEDURE. Seven healthy female dogs weighing between 13.5 and 24.0 kgm. were used for the clearance studies after they were thoroughly trained for lying quietly, catheterization and arterial puncture. The basal diet consisted of NaCl, one part of butter and four parts of starch. The amount was adjusted to furnish twice the calculated basal energy requirement. This diet is markedly deficient but since it was fed for no longer than five weeks at any one time it was considered satisfactory for these experiments. The essentials of Van Slyke's technique (6) were followed in determining the urea clearance. Three collections were made over three successive periods of about thirty minutes'

duration. When the urine flow was less than 0.4 cc. per minute the sample was discarded. The hypobromite method (7) of urea analysis was used except when the dogs were on the high protein diet. Under the latter condition the hypobromite method gave results for blood urea 100 per cent or more above that of the urease method. Under the other conditions of our experiments the hypobromite and urease methods checked.

After the dogs were on the basal diet for about ten days the experimental study began. The clearances were made about five hours after the last feeding. The test substance was added to the basal meal in amounts corresponding to 0.0032 mol per kilo of body weight. Increasing the

TABLE 1

Number of urea clearances and the range of the percentage of increase above the basal clearance

	NUMBER OF CLEARANCES	RANGE OF PERCENTAGE INCREASE*
Basal (starch and butter)	60	
Glycine	12	46 to 59
d-l-alanine	6	8 to 57
Glycollic acid	10	10 to 45
Lactic acid	8	20 to 45
Pyruvic acid	8	13 to 60
Acetic acid	15	23 to 74
Propionic acid	16	38 to 75
Gluconic acid	15	0 to 9
Benzoic acid	12	0 to 71
Basal (starch and Crisco)	34	
Butter (50 grams)	27	14 to 81
Crisco (50 grams)	27	0 to 14
Non-saponifiable matter of butter	12	0 to 58
Non-saponifiable matter of butter and 50 grams of Crisco	8	34 to 111

* The percentage of increase in clearance produced by the test substance was calculated from the immediately preceding basal clearance.

dosage resulted in higher clearances so that our results are by no means maximal. In most cases the basal clearance was ascertained on the same day as that with the test substance. Since the basal clearance remained fairly constant from day to day, it was not always determined just before the test substance was tried, but was ascertained every four or five days. Clearances are expressed in cubic centimeters of plasma freed of urea per minute per square meter of body surface. The surface area was calculated by the formula of Cowgill and Drabkin.

For the sake of conciseness, the clearances obtained on all the dogs after ingestion of the various carboxylic acids are plotted in figure 1. The

basal clearances present a wide variation because some dogs consistently showed a higher basal clearance and also, the basal clearance generally fell over the two or three weeks that the test substances were studied. This explains why the data of figure 1 do not seem, on casual inspection, to give the percentages of increase shown in table 1. The percentage of

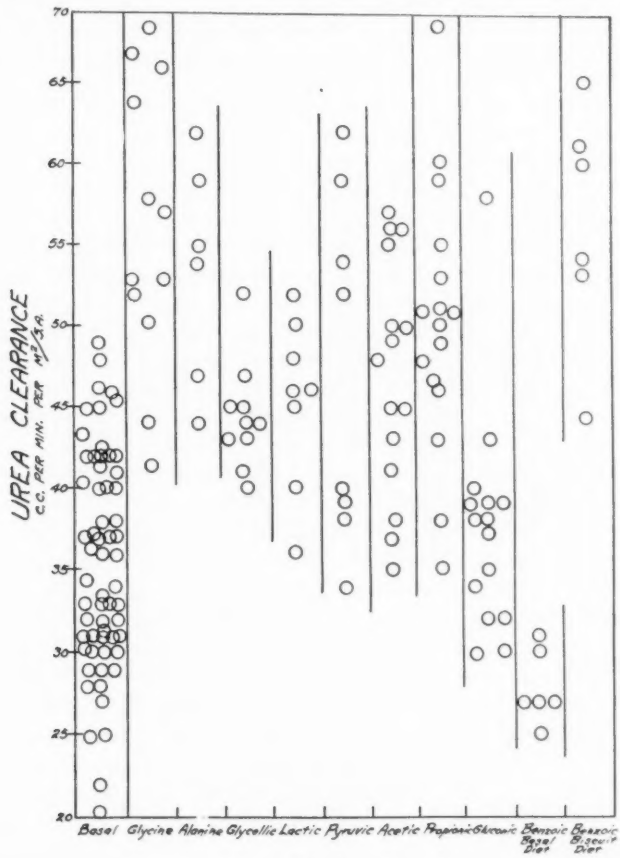

Fig. 1

increase caused by the test substance was calculated from the preceding basal clearance and not from any average of basal clearances.

Results. The data as presented in table 1 and figure 1 show that all of the aliphatic acids except gluconic gave the same qualitative effect as did the amino acids, although glycine did seem to exert greater stimulation than glycollic acid. The effectiveness of ingested lactic acid sug-

gests an explanation as to why moderate exercise provokes a diuresis (8). Benzoic acid seemed to depress the clearance when the basal values were low but showed marked stimulation when given to dogs on a biscuit diet.

After finding that fatty acids increased the urea clearance, we evaluated the effect of butter in the basal diet by its withdrawal, in one of the dogs, whereupon the basal clearance over a period of sixteen days decreased 22 per cent. With four parts of starch and one of Crisco as a basal diet, the relative effects of the ingestion of 50 grams of butter or Crisco were

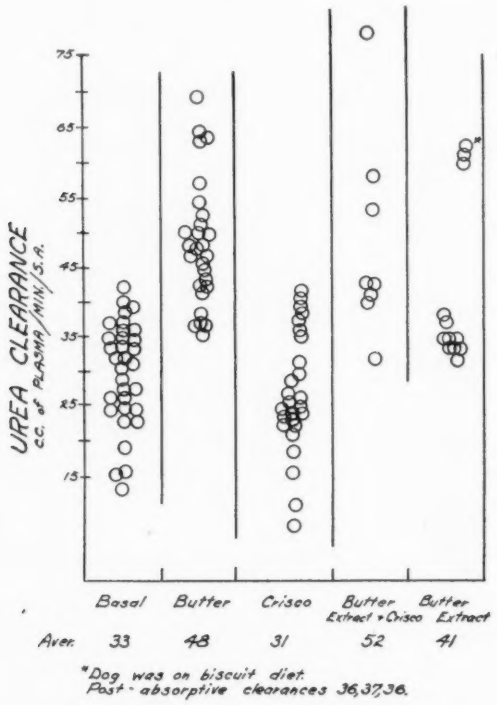

Fig. 2

compared and the results are presented in table 1 and figure 2. The average post-prandial clearance with butter was 45 per cent higher than the average of the basals in figure 2 whereas with the additional Crisco there was no significant variation from the basal clearance. An ethereal extract of the non-saponifiable matter of butter was prepared. An aliquot portion of this extract, corresponding to 75 grams of butter, was added to the basal diet of 50 grams of Crisco and the ether removed by warming on a water bath. This non-saponifiable matter with the basal diet

resulted in a 27 per cent increase in the average urea clearance and with 50 grams of Crisco it resulted in a 57 per cent increase in the average clearance. A blank extract made up of the reagents used in preparing the non-saponifiable extract was entirely ineffective.

Discussion. The brilliant contributions from the workers who have used the tissue-slice method to study metabolic problems afford a suggestive basis for a possible explanation of the effect of these various substances upon urea clearance. Krebs (9) found the kidney cortex to be the most actively deaminating tissue per unit of weight. The addition of amino acids to kidney slices resulted in a large increase in ammonia production and an approximate doubling of oxygen consumption. Elliott, Benoy and Baker (10) found with similar technique that lactate and pyruvate are removed partially by oxidation and partially by conversion to other acids. Jowett and Quastel (11) have reported that while kidney slices produce aceto-acetic in small quantities from added fatty acids such as butyrate, crotonate, hexanoate, etc., they also had the power to destroy aceto-acetic acid. In these experiments an R.Q. of less than unity even in the presence of glucose, suggested that the fatty acids were oxidized. Sodium benzoate was found by Jowett and Quastel (12) to inhibit aceto-acetic acid formation in rat liver but the inhibition decreased on ascending the fatty acid series.

These results with the surviving tissue slices suggest that in the intact animal the kidney might metabolize not only amino acids and fatty acids but also the metabolic derivatives of these substances which are produced by the metabolism of other organs such as the liver and skeletal muscle. The fact that gluconic acid, which is known not to be metabolized (13) within the body, failed to affect urea clearance, while the substances such as amino acids and fatty acids increased the clearance, at least suggests that metabolic factors might have some causal relation to the process.

The studies of other workers on the effect of diet upon urea clearance indicate that there is a corresponding increase in glomerular clearance. Jolliffe and Smith (2) and Shannon, Jolliffe and Smith (3) found that although increasing the daily protein intake from 9.6 grams to 180 grams increased glomerular and urea clearances more than 100 per cent, the urea clearance: glomerular clearance ratio remained rather constant. Pitts (5) stated that in his experiments the increased renal function could be explained in large part by increased glomerular filtration. It is conceivable that the products of the suggested metabolic processes might influence urea clearance either by affecting glomerular filtration or a circulatory factor.

That urea clearance might be increased by certain metabolic products is at least physiologically reasonable. It might serve as an adjustment

to the metabolism of the entire organism. It might make understandable the minor rôle of the nervous system in regulating renal activity (4).

Future experiments must ascertain the significance of the effect of the non-saponifiable matter of butter upon renal excretion.

SUMMARY

The finding of other workers that meat feeding markedly elevates urea clearance of dogs in the post-prandial and post-absorptive states above that for a low protein diet is confirmed. Casein, after repeated acid precipitation, is almost as effective per gram of protein as is meat.

Glycine, synthesized glycine, d-l-alanine and glutamic acid are very effective in elevating post-prandial urea clearance.

Lactic acid, pyruvic acid, glycolic acid and deaminated glycine are approximately as effective as the corresponding amino acids. Therefore, the effect of protein on urea excretion is not necessarily due to deaminization. Fatty acids such as acetic and propionic elevate urea clearance.

Gluconic acid, which is not metabolized by animal tissues, did not elevate urea clearance. Benzoic acid seemed to depress the clearance when the basal values were low but markedly elevated it when the clearance was at an average level.

Butter, when added to a meal of starch and Crisco, increased the average urea clearance 45 per cent, but additional Crisco had little effect. The non-saponifiable matter of butter when fed with starch and Crisco increased the average urea clearance 27 per cent and when fed with 50 grams of Crisco increased the average clearance 57 per cent.

These observations suggest the possibility that renal metabolism may in some way be related to renal excretion.

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EFFECT OF BATHS AT DIFFERENT TEMPERATURES ON OXYGEN EXCHANGE AND ON THE CIRCULATION

H. C. BAZETT, J. C. SCOTT, M. E. MAXFIELD AND M. D. BLITHE

From the Departments of Physiology, University of Pennsylvania, and of Hahnemann Medical College, Philadelphia

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The experiments here reported were undertaken to test further the general applicability of the empirical equations for the calculation of cardiac output from blood pressure and pulse wave velocity measurements (1935). Baths were employed, since in this way great variations in vaso-motor tone and pulse rate, as well as of body temperature, were attainable. To render the equations useful in clinical conditions, it is essential to show that they are valid in conditions where these functions are abnormal.

METHODS. Emphasis has been placed throughout on the comparison of estimates of the cardiac output obtained by calculation with those derived from the rate of acetylene absorption. As in previously reported experiments (1936) the two methods were used alternately.

For the acetylene estimations of cardiac output oxygen consumption was measured by the Sanborn clinical apparatus. Where the changing condition of the subject created difficulties, the value used was obtained by interpolation from estimates before and after the acetylene procedure. In the rebreathing procedure the reclining position of the subject coupled with the hampering of movements by the water made mixing in the lungs difficult, and in many cases, where the initial samples were taken relatively early, mixing was certainly incomplete, so that the estimates had to be discarded. Consequently, if samples were taken relatively early, the subject had to be propped up into a sitting posture, where thermal conditions were temporarily altered through evaporation. Most commonly two samples were taken after some 13 and 19 seconds respectively with the subject so propped up; in a few experiments adequate mixing seemed to be attained with samples taken at 11 and 16 seconds.

Estimation of cardiac output from the blood pressures followed the technic previously described; in the earlier experiments apex beat records were used to determine the start of ventricular ejection, in the later experiments sternal records (as described in 1936) were employed. It proved to be difficult to read diastolic pressure accurately in the hot

bath experiments. Under conditions of peripheral vasodilatation the diastolic criterion was much less clearly marked, particularly in young subjects. The development of a negative wave in the brachial pulse and the sharp upstroke of the oscillometric pulsation were found at compres-

TABLE 1

Comparison of mean values for cold baths, basal in air, baths at 35° and various stages of warm baths

(Means of individual subject averages)

CONDITION	O_2 CONSUMPTION	A V Diff.	C I (Acct.)	F	C I (Calc.)	S	D	R	PULSE WAVE VELOCITIES			
									1	2	3	4
	cc. per min.											
1. Cold baths Bath 32.1° Rectal 36.9°	213	62.7	2.14	57.4	2.07	114	69	130	3.02	6.39	7.22	8.9
2. Basal in air (in other experiments)	198	59.2	2.04	59.8	2.18	103	65	118	3.15	5.49	6.65	9.3
3. Neutral baths Bath 35.1° Rectal 36.9°	195	52.2	2.28	60.7	2.24	99	61	108	3.14	5.39	6.32	8.7
4. Warm baths early Bath 37.9° Rectal 37.6°	220	48.7	2.76	82.3	2.69	103	63	93	3.63	5.98	6.18	9.0
5. Warm baths middle Bath 38.8° Rectal 38.2°	246	53.5	2.81	91.8	3.47	108	59	78	3.99	5.78	6.56	8.9
6. Warm baths late Bath 38.6° Rectal 38.8°	253	58.6	2.73	91.2	3.18	110	59	86	4.15	6.09	6.71	9.7

F represents pulse rate, S and D estimates of lateral systolic and diastolic pressure. C I indicates the cardiac output per square meter per minute (expressed as an index, though this is normally only used for basal states). R is the calculated effective peripheral resistance, and pulse wave velocities 1 to 4 represent heart to subclavian, subclavian to femoral, subclavian to brachial, and femoral to dorsalis pedis velocities respectively.

sions which differed from one another to an unusual extent. The latter, however, gave consistent values if the records were read with great care with the assistance of a cylindrical lens.

The experiments were made on the same subjects that were used in experiments on meals (1936), and the subjects are therefore given the same

numbers. They were basal and were deprived of fluid; they were immersed in baths to their necks. The heating and regulating of the bath were arranged as described by Bazett and Burton (1936), except that the paraffin cover was omitted.

At the beginning of every experiment the bath temperature was regulated at 35°; later it was either cooled or warmed. The bath temperature was read from time to time, and the rectal temperature was recorded continuously by the use of a resistance thermometer (Leeds and Northrup type).

EXPERIMENTAL DATA. *Baths at 35°.* Observations were made in 25 experiments, and in all 4 subjects the conditions in baths at this temperature were very similar to those of the basal state in air. This is demonstrated in the average values shown in table 1. In the baths the arterial-venous oxygen (A-V) differences and the blood pressures were somewhat lower, as was also the calculated value for the effective peripheral resist-

TABLE 2

Individual values in neutral baths and differences of these values from those of the same subjects basal in air

SUBJECT	F	PER CENT CHANGE	S	PER CENT CHANGE	D	PER CENT CHANGE	R	PER CENT CHANGE	A-V	PER CENT CHANGE	RECTAL	SENSATION
4	77.5	−3.4	95.6	−1.6	62.1	−0.3	94	−2.6	53.0	−1.2	37.09	Cool
8	49.0	−0.6	104.0	−2.2	62.9	−2.4	123	−7.4	51.0	−11.8	36.70	Cool to neutral
14	63.3	+4.2	95.2	−3.6	55.5	−9.8	102	−10.8	52.4	(−19.9)*	37.04	Neutral
10	53.1	+8.1	102.5	−8.0	63.2	−10.6	114	−10.8	51.1	−12.6	36.92	Warm to neutral

* The number of observations on this subject basal in air were few.

ance (R). These changes are those to be expected in a slightly warmer environment. The peripheral pulse wave velocities were also slightly slower, and this may also have depended on the raising of the temperatures of the peripheral areas to a uniform surface temperature, which would occur in such baths. Though all the differences were slight, the changes in the average values are probably significant, since the degrees of change appeared to be correlated with the subject's sensation of temperature (rather than with the difference in temperature between the rectum and the bath). The individual values are shown in table 2; the contrast in the effects of the baths in subjects 4 and 10 on pulse rate, blood pressures and A-V oxygen differences, and the intermediate position of the other subjects, is so consistent, that it must be significant. The baths appeared cool to subject 4, where the rectal–bath temperature difference was high, as well as to subject 8 where this difference was low. Subject 8 was the thinnest subject; this was probably a factor.

The mean discrepancy of the cardiac outputs in baths estimated from the blood pressures from those determined by acetylene was ±14.7 per cent of the acetylene values.

Cool baths. Six experiments were made on the 4 subjects. Baths were cooled suddenly to 32°, occasionally to 31°. Baths below 32° were apt to produce shivering; this interfered with the records and such temperatures were consequently avoided. The mean values obtained are given in table 1. The oxygen consumption was usually increased considerably during the initial sudden cooling; after this it returned towards a normal value and occasionally became completely normal or even subnormal. The value in the table represents the average level during this later period. The cardiac output was usually reduced but was occasionally increased by both methods of estimation; the A-V difference was usually increased. The blood pressures and the value of R were always raised. The changes in pulse wave velocity were slight, though probably significant; an increase in the rate in the peripheral vessels accompanied cold, while the velocity from the heart to subclavian was reduced in spite of the rise in blood pressure. The pulse rate was slowed or showed no change; there was no significant change in the average values for stroke volumes. The following abbreviated protocols represent the data on which the mean values are based.

Protocol 1. X/12/34. Subject 4. Weight 43.1 kilos with no measurable weight loss during the experiment; at 9:30 a.m. entered bath at 35.1°C. The experimental period was divided into 4 stages: (a) 10:18 a.m. to 10:30 a.m. with bath at initial temperature of 35.1°C., rectal temperature 37.15°C.; (b) 10:30 a.m. to 10:50 a.m. when bath temperature accidentally fell slowly to 34.5°, rectal to 37.05°; (c)10:50 a.m. to 12:21 p.m. with bath at 35.1°, rectal temperature at 36.9°; and (d) 12:21 p.m. to 1:44 p.m., with the bath at 32°, the rectal temperature at first rising to 37.0° (by 12:45 p.m.) and then dropping to 36.7° (by 1:44 p.m.).

PERIOD	OXYGEN CONSUMPTION *	A-V Diff.	C I (C_2H_2)	F	C I (Calc.)	S	D	R	PULSE WAVE VELOCITIES			
									1	2	3	4
(a)	—	—	—	76	2.35	96	58	97	3.1	7.0	10.9	7.6
(b)	—	—	—	75	2.0	103	65	125				*
(c)	162	51.2	2.4						3.1	6.9	10.2	7.5
(d)												
12:23	284											
12:30	153											
12:37	180											
12:44		57.9	2.35									
12:48	187											
1–1:20				74	2.4	110	72	106	2.4	7.4	12.8	8.2
1:33	222											

* Pulse wave velocities assumed to be the same as in period (c).

In this and following protocols the oxygen values represent consumption per minute estimated from a 6 to 15 minute period. The various symbols used are the same as those of table 1. Values for blood pressure and pulse wave velocity represent the mean values of some 4 to 20 estimates.

The experiment demonstrates that a mere fall of bath temperature to 34.5° (b) greatly increased the blood pressure, though the cardiac output was probably not increased and may have been decreased; the pulse rate was slightly affected. The later fall of temperature (d) increased the A-V difference; oxygen consumption was high during the initial cooling, returned to a normal level, rose again ultimately when the rectal temperature had ceased to rise.

An example may be given where the oxygen consumption was reduced in the cold bath.

Protocol 2. IX/21/34. Subject 8. Weight 56.0 kilos, with no measurable weight loss during the experiment; at 10:00 a.m. entered bath at 35.2°C. The experimental period was divided into 2 stages: (a) from 10:33 a.m. to 12:26 p.m. with bath at initial temperature of 35.2°C., rectal temperature at 36.55°C.; and (b) from 12:26 p.m. to 1:32 p.m. bath temperature at 31.9°, rectal temperature at first rising to 36.61° (by 12.28) but after 12:50 p.m. falling to 36.28° by the end of the period.

PERIOD	O_2 CONSUMPTION	A-V Diff.	C I (C_2H_2)	F	C I (Calc.)	S	D	R	PULSE WAVE VELOCITIES			
									1	2	3	4
(*a*)	214	52.1	2.5	52	2.0	106	66	130	3.0	6.4	6.1	10.2
(*b*)												
Initial	214											
cooling												
12:43	185											
12:48		66.8	1.76									
12:56–1:29				45	2.05	126	79	144	2.95	6.2	5.5	9.2
1:13	207											

The two examples quoted demonstrate that conditions are not stable for oxygen consumption, nor probably for cardiac output, during such a period of cooling. In other experiments the oxygen consumption sometimes showed much greater increases, in one case amounting to 45 per cent.

The mean discrepancy between the cardiac output calculated from that estimated by acetylene was ±11.3 per cent of the acetylene value.

Warm baths. There were 18 experiments on the 4 subjects. The maximal increase in rectal temperature was 2.9°, the maximal level 39.8°, the minimal 1.3° and 38.65°. The rates of change varied between 0.6° and 3.6° per hour. The highest bath temperature was 39.5°. The weight loss varied between 1.3 and 5.2 per cent of the body weight; losses of the lower order occurred in the female subjects 4 and 14 and of the higher in the male subjects 8 and 10.

The bath temperature was sometimes allowed to rise gradually but more commonly was initially raised rapidly and was then kept constant. In both cases it was ultimately, with very few exceptions, regulated at a somewhat lower level, such as would maintain a constant rectal temperature (see fig. 4). In some experiments the two modes of heating were

combined. Except in a few cases, where the rate of rise was fast enough to induce hyperpnea, the early symptoms were mild and were only slightly exaggerated by the more rapid changes in temperature. In the later stages the subjects suffered from restlessness, prostration, and air hunger. After the bath subjects 4 and 10 recovered rapidly, except for prolonged thirst in the more dehydrated subject 10; subject 8 had in addition to the thirst a hoarse voice which lasted till the next day; subject 14 showed weakness and a tendency to faint after leaving the bath.

The average effects are shown in table 1. Some abbreviated protocols are also submitted to illustrate the types of reaction. These are chosen from experiments in which the rates of rise of bath and rectal temperature were slow, since these experiments were relatively uniform as far as the temperature changes were concerned, yet the type of response differed.

Protocol 3. VII/15/35. Subject 4. Weight 45.2 kilos with loss of 0.6 kilo (1.3 per cent); at 9:45 a.m. entered bath at 35.1°C. The experimental period was divided into 4 stages: (a) 10:10 to 11:30 a.m. with bath at its initial temperature and rectal 37.1°; (b) 11:30 a.m. to 1:00 p.m. with bath temperature rising gradually to 37.75°, and with the subject's rectal temperature rising at 0.68° per hour and exceeding that of the bath; (c) 1:00 to 1:47 with the bath temperature rising to 38.5°, with the rectal rising at 0.78° per hour to 38.35° and always below that of the bath; (d) 1:47 to 2:10 with bath temperature rising to 38.8° and the rectal to 38.65° at a rate 0.84° per hour.

PERIOD	OXYGEN CONSUMPTION	A-V Diff.	C I (C_2H_2)	F	C I (Calc.)	S	D	R	PULSE WAVE VELOCITIES			
									1	2	3	4
(a)	139	42.0	2.45	75	2.35	97	69	110	3.0	4.6	5.5	8.3
(b)	175	45.5	2.85	80	2.50	90	61	96	3.5	4.1	5.2	9.5
(c)	194	50.7	2.85	104	2.70	96	64	90	4.3	5.1	6.1	8.6
(d)	—	—	—	106	2.50	95	62	96	5.3	5.1	5.7	8.0

The stroke volumes were in period (a) 43 cc., in (b) 45 cc., in (c) 36 cc. (mean estimates) and in (d) 32 cc. (calculated estimate).

Protocol 4. XII/7/34. Subject 8. Weight 55.0 kilos with loss of 1.7 kilo (3.1 per cent); at 9:58 a.m. entered bath at 35.2°. The experimental period was divided into 3 stages; (a) 10:37 to 12:19 p.m. with bath at the initial temperature and rectal temperature 36.95°; (b) 12:19 to 2:03 p.m. bath temperature rising gradually to 38.85, and the subject's rectal temperature rising at 1.05° per hour to 38.45°; (c) 2:03 to 2:47 p.m. with bath dropped to 38.3° and then maintained steady, and rectal temperature rising at first and then remaining steady at 38.65°.

PERIOD	OXYGEN CONSUMPTION	A-V Diff.	C I (C_2H_2)	F	C I (Calc.)	S	D	R	PULSE WAVE VELOCITIES			
									1	2	3	4
(a)	192	45.8	2.50	48	1.95	95	58	119	2.8	5.7	7.2	10.0
(b)	226	53.0	2.60	82	2.75	111	61	95	4.0	5.8	7.5	8.3
(c)	253	66.6	2.30	95	2.10	129	77	140	4.4	5.5	7.7	13.0

During period (c) the pulse rate was progressively rising and at the end of the period was 102; the cardiac output was reduced by both methods of estimation; the stroke volume was lowered from 71.5 cc. (mean estimate) in period (a) to 38.5 cc. in period (c).

Protocol 5. VII/2/35. Subject 8. Weight 56.7 kilos with loss of 1.8 kilo (3.2 per cent); at 9:30 a.m. entered bath at 35.2°C. The experimental period was divided into 4 stages: (a) 10:12 to 11:58 a.m. with the bath at its initial temperature and rectal 36.7°; (b) 11:58 a.m. to 1:22 p.m. with the bath temperature rising gradually to 38.2° and with the subject's rectal rising at 1.35° per hour to 37.7°; (c) 1:22 to 2:06 p.m. with bath temperature rising very slowly to 38.6° and the rectal rising at 1.35° per hour to 38.6; (d) with the bath temperature dropped to and maintained at 38.5°, and rectal rising at 0.48° per hour to 38.9°.

PERIOD	OXYGEN CONSUMPTION	A-V Diff.	C I (C_2H_2)	F	C I (Calc.)	S	D	R	PULSE WAVE VELOCITIES			
									1	2	3	4
(a)	204	58.0	2.05	48	2.20	104	62	113	3.3	4.7	5.8	8.6
(b)	228	61.5	2.20	68	4.15	115	56	64	3.7	5.4	7.1	8.5
(c)	—	—	—	82	5.05	125	60	55	3.6	6.2	8.0	9.1
(d)	276	65.1	2.55	82	4.75	127	58	61	3.9	6.7	8.6	9.3

The stroke volume in period (a) was 75.5 cc. (mean estimate); in period (d) it was by acetylene 52.0 and by calculation 95.5 cc.

These examples show good agreement between the two methods of estimation of *cardiac output* in protocols 3 and 4, marked disagreement in protocol 5. Such marked disagreements were not dependent on chance experimental error, for they were never observed in subjects 4 and 10, though obtained consistently in subjects 8 and 14 in experiments made during the summer months. During the winter these discrepancies disappeared, but reappeared the following summer. They were not seen except when the calculated cardiac indices exceeded 3.4 nor marked unless they exceeded 4 liters. In determining the mean discrepancies between the two estimates the data obtained during the summer months are excluded except for data obtained very early during heating, and the discrepancy is then ± 12.9 per cent of the acetylene value (13 comparisons). In the summer experiments on subjects 8 and 14 the mean discrepancy was 52.1 per cent in 10 comparisons, with the calculated values always the greater; such experiments account for the discrepancies seen in the last two conditions of table 1. In summer experiments on subjects 4 and 10 on the other hand the mean discrepancy was only ± 10.4 per cent, though it is significant that in 6 of the 7 comparisons the calculated values were the higher.

The individual comparisons of the two estimates are shown graphically in figure 1. While there is considerable scatter, no consistent discrepancies are disclosed except in the summer data with warmth (indicated by triangles); these are detectable in both pairs of subjects, though barely so in subjects 4 and 10.

The absence of consistent discrepancies in the earlier stages of heating, whether in summer or winter, is indicated by the composite data of figure

2, which were obtained by averaging the means of the values for individual subjects. This graph also illustrates the physiological changes observed, and the mean time intervals of their occurrence. Such mean values, however, mask some of the physiological changes, since summer and winter values differed in several respects. Consequently some of the data are regrouped into summer and winter means in table 3, and

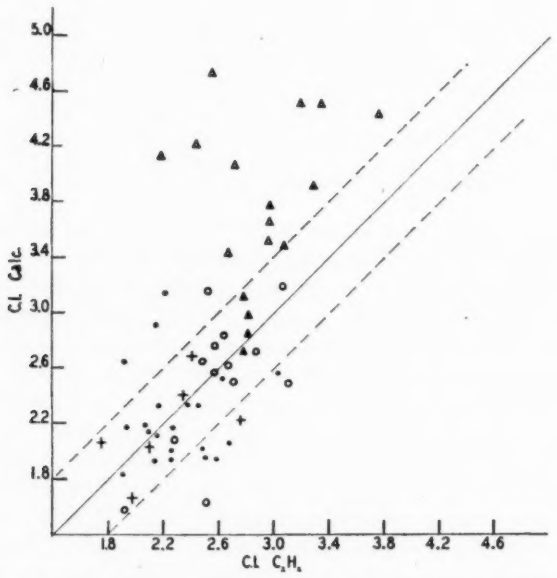

Fig. 1. Calculated cardiac outputs per square meter per minute as ordinates plotted against acetylene estimates as abscissae. Values obtained in cold baths are indicated by crosses, in neutral baths by closed circles, in warm baths in winter experiments and very early observations in similar experiments in the summer by open circles. Late observations in warm baths in the summer are indicated for subjects 4 and 10 by closed, for subjects 8 and 14 by open triangles. The lines drawn represent the theoretical correspondence and the limits within which the data should fall if the error of each estimate did not exceed ±0.2 liter per sq. m. per minute; 70 per cent fall within these limits if the summer data on subjects 8 and 14 are excluded.

such differences, as are considered significant, are indicated in the table in italics. The final periods of heating differ in the winter from those in the summer by showing a higher A-V difference, a greater reduction in stroke volume by both methods of estimation, a greater increase in pulse rate, a smaller increase in pulse pressure, and a rise instead of a fall in diastolic pressure. All these changes are such as might accom-

pany commencing circulatory failure as the result of dehydration, yet in the winter experiments the temperature changes were slightly less, and the weight losses also lower.

Though there was agreement between the two methods in these differences, the magnitude of the effect was estimated quite differently. Acetylene indicated a reduction of stroke volume on warming both in the

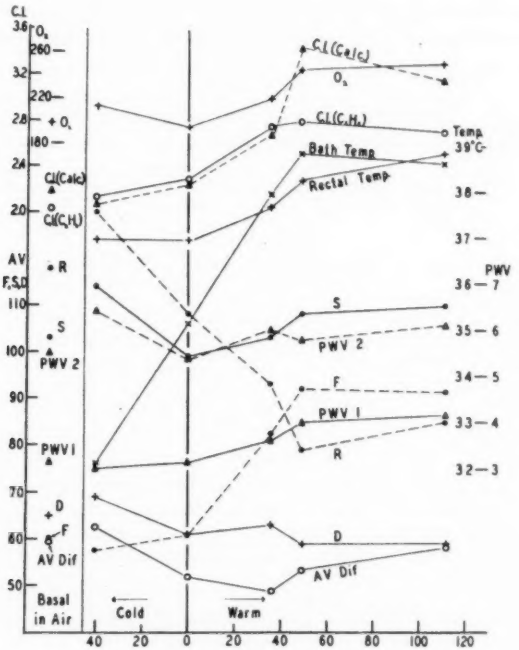

Fig. 2. From a central zero representing neutral baths at 35° the data obtained, in cold baths to the left and in warm baths to the right, are plotted in relation to time in minutes as abscissae. The values are all composite mean values. Similar means for the subjects basal in air are indicated on the extreme left. For symbols see table 1.

summer and winter except for two experiments in the summer where a temporary increase appeared to be present; one of these two experiments is quoted in protocol 3. On the other hand the stroke volumes calculated from blood pressures usually appeared to be increased slightly in the period of rising temperature during the summer, and to be reduced later on the average only to normal values, while in the winter the stroke volumes were definitely subnormal in both stages. To account for such

discrepancies one of the methods must show a systematic error in the summer experiments; the probable real changes in cardiac output consequently will be discussed later in relation to the validity of the two methods.

The *oxygen consumption* increased as the body temperature rose, and approximate calculation of the value of the temperature coefficient Q_{10} was possible, since the changes were considerable. Comparison has been made with the data obtained in baths at 35°, since those obtained at lower temperatures were complicated by a variable metabolic response to cold (see Burton and Bazett, 1936), while the thermal gradients with

TABLE 3

Comparison of summer and winter mean values

Summer experiments (duration 138 minutes); winter experiments (duration 126 minutes)

	SUMMER			WINTER		
	Bath at 35°	Period of rising temperature	Final period (rectal 38.86°)	Bath at 35°	Period of rising temperature	Final period (rectal 38.72°)
Oxygen usage per square meter	120.5	149.5	154.5	119.0	140.5	153.0
A-V difference (cc. per liter)	54.2	50.0	*51.5*	53.6	53.8	*66.3*
Stroke volume (Acet.) (cc. per sq. meter)	37.6	32.6	*31.8*	42.0	32.4	*26.9*
Stroke volume (Calc.) (cc. per sq. meter)	37.6	*41.7*	*37.0*	36.5	*29.2*	*26.3*
Pulse rate	61.0	89.0	*91.5*	59.8	90.6	*96.2*
Systolic pressure (S)	100.9	108.9	110.6	95.7	107.9	110.6
Diastolic pressure	61.7	*57.4*	*56.1*	59.4	*62.0*	*66.2*
Pulse pressure (lateral)	39.2	*51.5*	*54.5*	36.3	*45.6*	*44.4*

In this table all the data are grouped into two periods, one of rising temperature, and the other, when the rectal temperature was nearly constant and the subject was dehydrated, in the ultimate stage of the experiment.

the subjects in air were less uniform. Values so calculated for Q_{10} vary so much that the rectal temperature must provide a very inadequate indication of the changes in mean body temperature, but more consistent and more probable values are obtained if comparisons are made on the basis of average body temperature as estimated from surface and rectal temperatures according to the formula developed by Burton (Burton and Bazett, 1936). If the average data of periods 3 and 6 of table 1 are compared, the values calculated for Q_{10} are 3.95 on a rectal temperature and 2.95 on an average temperature basis. In these periods the subject was approximately in a steady state; if periods 4 and 5 are considered, where this was not the case, Q_{10} values of 5.6 and 6.9 are obtained on a rectal, of 2.3 and 3.2 on an average temperature basis. Even

the individual data agree with a value for Q_{10} of about 2.9, when hyperpneic reactions did not complicate the issue. The complete data on subject 8, in whom such reactions were minimal, are shown in figure 3; they fall close to a curve for a Q_{10} of 2.9, if calculations are made on an average temperature basis, though on rectal temperatures values falling between 2.1 and 10.3 with a mean of 5.0 may be calculated. In other subjects, with more irregular oxygen consumption accompanying hyperpnea, occasional individual Q_{10} values may exceed 6.5 even on an average temperature basis, while calculated from rectal temperatures they may exceed 69.0. During the hyperpneic response to rapidly rising temperatures there appears to be some factor which increases oxy-

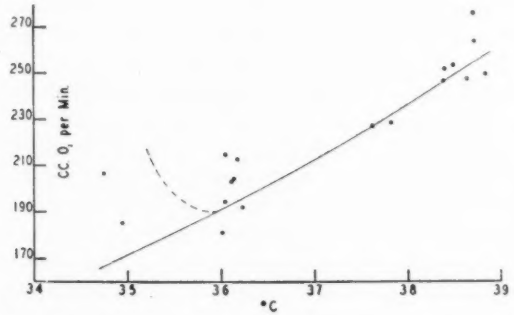

Fig. 3. Oxygen consumptions in cubic centimeter per minute are plotted as ordinates against estimated average body temperatures as abscissae. The points represent all the data in both cold and warm baths for subject 8. The line represents the theoretical values on an average subject if $Q_{10} = 2.9$, and its dotted prolongation the probable divergence from this line in an average subject in response to sensations of cold. The extreme values for Q_{10} for the individual estimates for subject 8 were 1.8 and 4.5 with an average of 2.88.

gen consumption (Bazett, 1927), and which gives values which are not readily explicable on a Q_{10} basis as the result of errors in estimating average body temperature.

The changes in *arterial-venous oxygen differences* are indicated in tables 1 and 3 and in figure 2. A reduction in the A-V difference was commonly seen especially in the early stages of the summer experiments. On the other hand there were undoubtedly very high A-V differences present in the terminal stages of winter experiments (reaching in one case on subject 10 to 82.5, a value established by the use of multiple samples). In the middle stages of an experiment when the A-V differences were low (or occasionally high as in protocol 5) the values, whether low or high, are of doubtful accuracy. This will be discussed later.

The *blood pressure changes* are indicated in tables 1 and 3 and in figure 2. As with A-V differences the changes in diastolic pressure differed in the summer and winter. In both seasons there was a tendency to an initial fall in diastolic pressure, but this was often reversed in the later stages, particularly in the winter experiments (table 3 and protocol 4). Systolic pressures might change in either direction but more commonly rose, especially in the later stages (table 3 but contrast protocol 3). Pulse pressures were increased by warmth, even when, as in winter experiments, there could be little doubt that the stroke volume had been decreased; the increase in pulse pressure was, however, not so great in the winter experiments where the decrease in stroke volume was marked. The increase in pulse pressure accompanying a decrease in stroke volume appeared to be correlated with a decrease in the distensibility of the large vessels, as was indicated by an increase in the pulse wave velocity in these vessels (fig. 2). This increase in pulse wave velocity was particularly marked in the winter experiments (compare protocols 4 and 5), and could not be correlated with changes in blood pressure level for it might occur with either a fall or rise in blood pressure (protocols 3 and 4).

The *pulse rate* was raised in warm baths. The changes on warming, like those on cooling, preceded changes in rectal temperature and cannot be explained as entirely due to changes in the temperature of the heart, though such changes may be an important factor in the later stages. But the increase in pulse rate on warming did not depend solely on surface temperature, as may be seen in figure 4, which shows the relation of pulse rate changes in two experiments to both bath and rectal temperatures. Even in the later stages it did not depend solely on rectal temperature, for the pulse rate was faster during heating than at the final higher plateau of rectal temperature (table 1 and fig. 4). In the final stages of an experiment with the onset of symptoms of marked dehydration the pulse rate was accelerated (fig. 4 and protocol 4).

Discussion. *The validity of the cardiac output estimates.* The data presented indicate that the circulatory changes induced by hot baths are not identical in the summer and winter months, though the differences are least marked in estimates of cardiac output by acetylene. Even by this method, however, higher cardiac outputs are indicated in the summer experiments, for cardiac indices of 2.7 or more were obtained in 13 out of 17 summer estimates, while indices of 2.7 were only exceeded on 4 occasions on exposure to heat in the winter. Since the possibility of error from recirculation in the acetylene method is admittedly a function of the circulation time and therefore of cardiac output, it is justifiable to exclude first from consideration the data obtained in the summer, when the cardiac outputs were high. If these be excluded, good agreement is seen between the two methods of estimation, as is indicated by figures 1

and 2, but there is a considerable scatter of the data and chance errors appear to be considerable. This is not surprising since the two estimates could not be made simultaneously and the subject was never in a steady state, while the chance error of either method may readily amount to ±10 per cent. Even in the preliminary baths at 35° the subject had to be propped up and exposed to new temperature conditions during the acetylene procedure. The discrepancies may well depend partly on physiological variables, and the absence of any systematic error may be accepted.

The validity of the acetylene estimates for varying cardiac outputs when the period of sampling extended to 19 seconds has been put to the

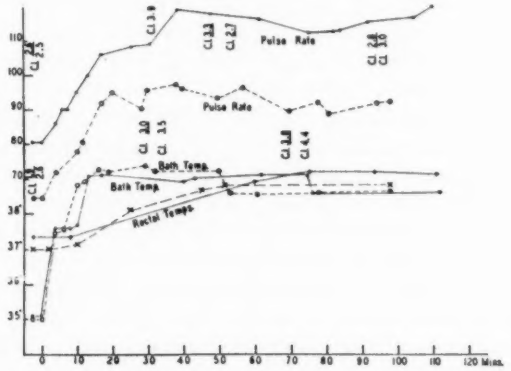

Fig. 4. The pulse rate changes in two summer experiments are plotted in relation to bath and rectal temperatures. Abscissae indicate the time in minutes from the commencement of warming. The solid lines represent an experiment on subject 4, the dotted lines one on subject 14. Bath temperatures are indicated by the lower curves with closed and open circles, rectal temperatures by crosses. The cardiac indices of subject 4 are indicated above and of subject 14 below; underlined values were obtained by acetylene, the others by calculation.

test by the use of multiple samples, as suggested by Grollman. Some of these data have been already presented (Scott et al., 1935), and indicate that no serious error is introduced within 24 seconds when the cardiac index is 2.2 or less, but that the critical time lies between 19 and 24 seconds with indices slightly exceeding 2.5. In the data there reported for indices below 2.2 the mean values were respectively 1.96 and 1.88 for sampling durations of 19 and 24 seconds, for indices above 2.5 they were 2.65 and 2.24. A duration of 19 seconds appears to be within the critical period for indices below 2.8, for in recent winter experiments samples have been taken after 11 and 16 seconds and compared with others ob-

tained after 14 and 19 seconds of rebreathing. Four such comparisons have been made; the two pairs of samples have been obtained within 15 minutes and the sequence of the tests varied. The mean indices obtained were for the shorter intervals 2.79, for the longer 2.76 and the values calculated from the blood pressures were 2.74. For still higher outputs such times must be too long, and if a high output is associated with a considerable cutaneous circulation and low A-V differences the error introduced by recirculation must make the A-V differences approach the normal, so that there may be no indication that a gross error is present. This has been the case in the longer time intervals, where errors have been evident, both in our data (Scott et al., 1935) and in those of Gladstone (1936). The latter worker finds evidence of appreciable errors even within 12.5 seconds in the high cardiac outputs of hyperthyroidism. The evidence of Christensen et al. (1933) that high outputs following exercise may apparently be correctly estimated by acetylene is not valid in this connection; after exercise oxygen consumption is high, and decreased acetylene absorption may be balanced by decreased oxygen intake as oxygen tension falls; analyses of alveolar air are not given, so that this balancing of errors cannot be excluded. In the high outputs of pyrexia, with a fast skin circulation, such balancing is less likely to occur.

The levels of the A-V differences and cardiac outputs in the earlier stages of heating, particularly in the summer, are therefore open to question. If the outputs were as high in the summer as those indicated by calculation (attaining an absolute value of about 8 liters per minute in protocol 5) the acetylene procedure used cannot be considered reliable, and gross overestimates of the A-V differences and under-estimates of the cardiac output may have been made. In favor of such an hypothesis is the fact that the lowest A-V differences and highest cardiac outputs were observed in the few summer experiments in which the sampling periods were kept within 16 seconds. The differences in the blood pressure and pulse rate pictures also throw doubt on the apparent constancy of the stroke volume in the period of rising temperature that is indicated by acetylene in the comparison of summer and winter experiments (table 3). The high outputs with an actual increase in stroke volume indicated in the earlier periods of heating by calculation from the blood pressures are therefore considered tentatively the more probable.

The changes observed in the winter are more certain; here not only are the results of the two methods in agreement, but also the acetylene values may be confirmed by triple sampling. In the summer such triple sampling is more apt to indicate the uncertainty of the values obtained than to establish their accuracy. The difference presumably depends on a real low cardiac output in the winter, one well within the range of the acetylene method. The high A-V differences of protocol 4 (c) were so estab-

lished and are included in the report (Scott et al.) under the heading of subject 3—pyrexia. Similarly the high A-V differences of 82.5 to which reference has already been made was reported under the heading of subject 4—pyrexia; it was obtained in the terminal stages of an experiment accompanied by great restlessness and prostration. Even in subjects 4 and 14, in whom there was little dehydration, higher A-V differences were observed in the winter, though a value of 60 cc. per liter was only once exceeded; in contrast in the summer months the values in these subjects often remained below the initial level. There can be no doubt that in the final periods a fall in cardiac output and rise in the A-V difference develops; this is probably dependent on dehydration which is less readily endured in the winter than in the summer.

The circulatory changes produced by the baths, as estimated on the above assumptions of validity, may be briefly discussed. On immersion in water at a neutral temperature no change in cardiac output is produced through any hydrostatic effect altering venous return, as was suggested by Tigerstedt (1917). Exposure to cold causes peripheral arteriolar constriction, increases the effective resistance, and raises blood pressure. A metabolic reaction follows if the stimulus is adequate, and, where the metabolism is increased, blood flow tends also to rise, though oxygen utilisation is increased. The pulse is slowed reflexly, either directly from sensations of temperature or secondarily as the result of the blood pressure changes; later changes in blood temperature may also affect it. Peripheral arteriolar constriction appears to be associated with dilatation of the large arterial trunks as judged by pulse wave velocities.

Exposure to warmth dilates the peripheral arterioles and lowers the effective resistance and tends to lower mean blood pressure. The latter change is, however, antagonised by an increased cardiac output, which is achieved mainly by an increased pulse rate, though in the summer there may also be an increase in stroke volume. Systolic pressure may be raised, and diastolic lowered; if the pulse rate increase is very great, the latter change is antagonised. In the later stages of heating dehydration occurs and is accompanied by restlessness, a sign of incipient circulatory failure. In attempting to compensate for this condition, in which the low cardiac output is quite out of proportion to the high oxygen consumption, the effective peripheral resistance is increased and there appears also to be constriction of the large arterial trunks as indicated by increased pulse wave velocities (compare the adjustment to splanchnic dilatation, 1936), so that the blood pressure levels are maintained or even raised. Oxygen utilisation is increased and high A-V oxygen differences may be recorded; if one assumes that the circulation through the skin is still maintained at a high level, these values must imply the mixing of this blood with highly unsaturated blood from deeper areas.

There can be little doubt that it is possible for the stroke volume to be decreased some 30 to 50 per cent with no decrease in pulse pressure, or even with an increase of over 20 per cent in this pressure. Pulse pressure is apt to be misleading in regard to stroke volume unless the distensibilities of the vessels are taken into consideration. Division of the pulse pressure by diastolic pressure (Stone, 1915), by mean pressure (Liljestrand and Zander, 1928) or by the square of the systolic pressure (Bazett, 1919) all fail to provide any adequate correction for this factor.

The changes observed are similar in many respects to those described by others, but the marked fall of diastolic pressure is not found; the changes in this pressure were however similar to those observed in anesthetised dogs by Cheer (1928). The cardiac failure following hyperpnea described by Davies and Holmes (1930) was not found, but the conditions for its occurrence were rarely present. The rapid pulse wave velocities at high temperatures did not appear to depend on the more rapid conduction of waves, which had a rapid upstroke (Ranke, 1934), for the changes were often opposite in direction in central and peripheral vessels (protocols 1 and 4 and table 1). The suggestion of Böger and Wezler (1936) that contraction of a vessel makes it more distensible, appears impossible; if this were true, cold must cause relaxation of the brachial and other peripheral large arteries and constriction in the more central aorta (table 1), a most improbable contingency.

The pulse rate changes appear to be mainly reflex in origin, as was suggested by Benson (1934), but they are certainly not determined by the surface temperature alone, nor is it necessary for the surface temperature to exceed that of the rectum (protocol 3), for an effect to be produced. The final result must certainly be modified not only by blood temperature but by reflex effects from the arterial and venous pressure levels. This view as to the relative importance of deep temperature changes and reflex effects reverses the view expressed earlier by Bazett (1931).

SUMMARY

1. Comparisons have been made between estimates of cardiac output by the acetylene procedure and by calculation from blood pressures and pulse wave velocities, when the subjects were immersed in cool, neutral or warm baths. In spite of marked changes in the vascular conditions the two methods gave results which indicated the same picture and did not differ from one another on the average by more than ± 14.7 per cent, provided that experiments made in the summer months on two subjects be excluded. Normal agreement was obtained in these subjects in the winter.

2. Such systematic discrepancies were only seen when the cardiac outputs estimated from the blood pressures exceeded 3.4 liters per square

meter per minute; it is argued that the cardiac outputs were probably high, and beyond the capacity of the acetylene method with the sampling times used.

3. On such interpretations the cardiac output might be slightly increased or decreased with a slowing pulse in cool baths. It was considerably increased with a larger stroke volume and increased pulse rate in the early stages of warm baths and was reduced to a basal level in spite of a high oxygen consumption in the later stages of warm baths as the result of dehydration. In this latter stage the stroke volume was very small. The subjects were much more susceptible to such dehydration in the winter, so that the initial increase in cardiac output was less marked, and any increase in stroke volume rarely demonstrable.

4. In the terminal stages of winter experiments with warm baths quite high A-V differences (up to 82.5) might be found. In view of the high skin circulation, these must have implied very low saturations in active tissues.

5. In warm baths both systolic and diastolic pressures may be lowered initially, but the fall in diastolic pressure is far less than those commonly described. During incipient circulatory failure following dehydration both pressures tend to be raised.

6. The large arterial trunks show properties which imply their utilisation as a blood reservoir; the pulse wave velocity in the ascending aorta is slowed in cool baths when the peripheral arterioles are constricted, quickened in warm baths when these are dilated, and becomes still more rapid as the individual attempts to compensate for dehydration.

7. The pulse pressure may be considerably increased, even when the stroke volume is decreased; such changes depend on decreased distensibility of the large arterial trunks.

8. Pulse rate changes to temperature are not explicable as the simple effect of either surface or rectal temperature changes; the factors involved are complex.

9. The increased oxygen consumption with rise in body temperature cannot readily be related to changes in rectal temperature; if, however, it be compared to changes in average body temperature the data give a value for Q_{10} of about 2.9.

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THE EFFECT OF ASPHYXIA ON MAMMALIAN A NERVE FIBERS

JØRGEN ERIK LEHMANN[1]

From the Laboratories of The Rockefeller Institute for Medical Research, New York

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Although the effect of asphyxia on nerve activity has been the subject of many previous studies, investigation of some of the points is again necessary because of the recent extension of our knowledge of the configuration of the after-potentials of mammalian A fibers by Gasser and Grundfest (1936), and because of the need for experiments in which the other variables affecting a nerve's behavior (Lehmann, 1937a, b) are under control at the time at which the oxygenation is varied. In the course of the investigation it became apparent that variations of activity occasioned by changes in oxygen tension, hydrogen ion concentration and salt balance depend upon mechanisms which are closely interwoven.

METHODS. The methods used were those described in a previous paper (Lehmann, 1937a). The nerves (phrenic, saphenous and peroneal nerves of the cat) were mounted in a moist chamber at 37°C. and kept at pH 7.45 through the employment of "complete" Krebs' solution (Lehmann, 1937a) in equilibrium with a 5 per cent mixture of CO_2 in oxygen. Asphyxiation was accomplished by substituting for the latter a 5 per cent mixture of CO_2 in nitrogen. No attempt was made to remove the small amount of oxygen (about 0.1 per cent) from the gas mixture as the experience of previous observers has shown that this amount of oxygen does not make the asphyxiation time longer than it is with pure nitrogen. The rate of flow of the asphyxiating mixture was sufficient to bring the oxygen tension to a low value in a few minutes.

RESULTS. *Changes in the action during asphyxiation. The spike* decreases in size and finally all signs of a response disappear. The time of extinction depends upon the previous history of the nerve. On a first asphyxiation, extinction occurs regularly in 25 to 35 minutes (table 1); on a second asphyxiation, as other observers have found (Fillie, 1908; Gottschalk, 1914, 1919), the time is shorter; and on a third asphyxiation it is still shorter. This shortening must be attributable to an effect produced in the nerve by the procedures, as nerves kept for an equivalent period without experimentation require the usual time of asphyxiation for extinction of the response. Some deficit must remain even when sufficient

[1] Fellow of the Rockefeller Foundation.

time is allowed after each asphyxiation to permit restoration of a normal configuration to the after-potentials. A change in the nerve analogous to that produced by a previous asphyxiation can be brought about by a temporary shift of the reaction of the nerve to pH 8.0. After 5 to 10 minutes at pH 8.0 and a return to pH 7.4, asphyxiation to the point of

TABLE 1

The table shows the time required for extinction of the spike and the after-potentials in the phrenic, saphenous and peroneal nerves (cat)

EXPERIMENT	NERVE	AGE	TEMPERATURE	AFTER-POTENTIALS		SPIKE	REMARKS
				Disappeared after	Returned to normal after	DISAPPEARED AFTER	
			°C.				
1	Phrenic	2 hrs.	37.0	10 min.	50 min.	27 min.	
2	Phrenic	5 hrs.	36.9	10–15 min.	35 min.	> 22 min.	
3	Phrenic	3 hrs.	37.0	6 min.	> 2 hrs.*	30 min.	
4	Phrenic	1½ hrs.	36.8	10–15 min.	> 35 min.	30–35 min.	
5	Phrenic	10 min.	36.8	13 min.	> 35 min.	35 min.	
6	Phrenic	9½ hrs.	37.8	8 min.		28 min.	
7	Saphenous	5½ hrs.	37.0	5 min.		25 min.	
8	Saphenous	1 hr.	37.0	15 min.	> 1½ hrs.	30–35 min.	
9	Saphenous	1 hr.	37.5			28 min.	
10	Peroneal	10 min.	37.0	12 min.	70 min.	25 min.	
11	Peroneal	3½ hrs.	37.4	11 min.	3 hrs.*	25 min.	
12	Peroneal	20 min.	37.3	8 min.	> 25 min. < 1 hr.	30 min.	
13	Peroneal	3 hrs.	37.2	8 min.	> 60 min.	35 min.	
	pH shifted to 8.0 and back to 7.4 before asphyxiation						
14	Saphenous	2 hrs.	37.5	4½ min.		10 min.	
15	Saphenous	1½ hrs.	37.1	10 min.		16 min.	
16	Saphenous	½ hr.	37.5			20 min.	
17	Peroneal	20 min.	37.6	11 min.		18 min.	
18	Peroneal	15 min.	37.5			23 min.	
	Repeated asphyxiation						
19 a	Peroneal	25 min.	37.4	8½ min.		28 min.	1st asphyxiation
19 b	Peroneal	2½ hrs.	37.0	7½ min.		13 min.*	2nd asphyxiation
19 c	Peroneal	3½ hrs.	37.0	5½ min.		8 min.	3rd asphyxiation
20 a	Phrenic	1 hr.	36.0	6 min.		30 min.	1st asphyxiation
20 b	Phrenic			7 min.		14 min.	2nd asphyxiation

* Supernormal spike height after asphyxiation.

extinction of the spike occurs in from 15 to 20 minutes instead of the usual 25 to 35 minutes (table 1).

The threshold. Raised thresholds in asphyxiated nerves have often been described. In addition there is also evidence for an initial period of lowered threshold. Heinbecker (1929) found lowered thresholds lasting during the first 15 to 30 minutes of asphyxiation in frog nerve, and Thompson and

Kimball (1936) found an analogous period in human nerves asphyxiated *in situ*.

During the first 8 minutes of asphyxiation the after-potentials in the phrenic and peroneal nerves resemble those found in nerves deprived of ionic calcium (Lehmann, 1937a, b). On the basis of the behavior of the after-potentials the threshold would be expected to fall, and this is what

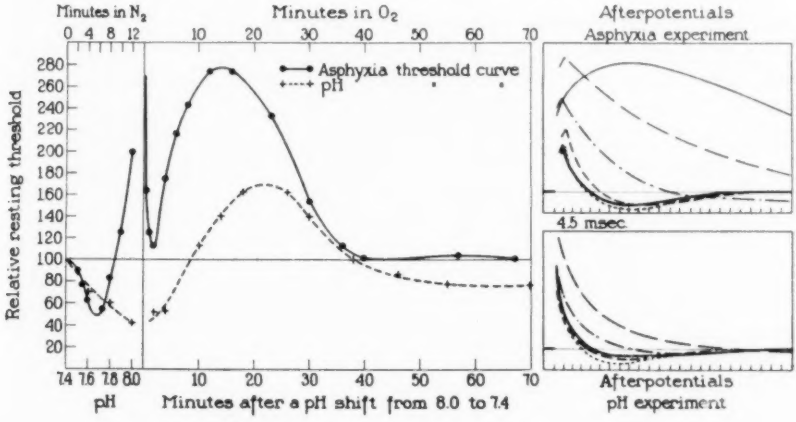

Fig. 1a Fig. 1b

Fig. 1a. Threshold curves from a peroneal nerve (cat) at 37°C. The dotted line shows the changes in threshold when the pH is changed from 7.4 to 8.0 and back to 7.4. The threshold at pH 7.4 is called 100. The abscissa at the bottom of the figure is in pH values during the period of alkalinization, and in minutes after the change back to the conditions giving pH 7.4 at equilibrium. The solid black line shows the threshold changes during asphyxia and during recovery in O_2. The time for this curve is at the top of the figure.

Fig. 1b. After-potentials recorded during the same experiment as in figure 1a. The lower part of the figure shows the after-potentials at different times after the change in pH from 8.0 to 7.4. The solid black line is the after-potential at 7.4 before the shift to pH 8.0. The other lines represent records after: 10 minutes —— ——; 18 minutes ——·——; 36 minutes ----------; 57 minutes ·····. The upper part of the figure shows the after-potentials during the recovery period after asphyxia. The records are taken at the same time intervals after readmission of O_2 as the records in the pH experiment after the pH shift. The thin line shows the rising phase in the after-potential and is recorded 2 minutes after readmission of O_2.

happens (fig. 1 a). The fall lasts for about 6 minutes and reaches a level which would be encountered in an oxygenated nerve at pH 8.0. Then the threshold begins to rise again; and it soon crosses the normal level to reach a stage at which responses can no longer be elicited. During the interval of low thresholds spontaneous firing occurs, the magnitude of the discharges varying with the excitability.

After-potentials. As other observers have found, the after-potentials disappear more rapidly than does the spike (5 to 15 min., mean 10 min., in this series). Another differential effect with respect to the spike is found in the fact that repeated asphyxiation makes little difference in the extinction time (table 1). More interesting than the effect of asphyxiation on the size of the potential, however, is its effect upon the form. As the latter changes in different ways in the several nerves which have been studied, the nerves must be described separately.

The changes in the after-potentials of the peroneal nerve are shown in figure 3. After 2 minutes of asphyxiation (fig. 3 a) the negative after-potential begins to decrease and the positive after-potential to increase (compare with the normal form in fig. 2 a). After 5 minutes (fig. 3 b) all negativity has disappeared, spontaneous discharge is marked, and the positive potential has a form resembling the mirror image of the normal negative after-potential. During the next 5 minutes the discharge and the positive potential progressively decrease (fig. 3 c) and after 13 minutes they are hardly visible (fig. 3 d).

During the first 5 minutes of asphyxiation the progression resembles very closely the changes produced by increasing the pH. For comparison the changes in the after-potential occurring in the same nerve between pH 7.4 and pH 8.0 are shown in figure 2.

In the saphenous nerve the picture is quite different. The decrease of negative after-potential and the increase of positive after-potential are very much less and little spontaneous discharge occurs. In extreme cases discharge is absent (fig. 5) and there is no significant positivity at any stage. The phrenic nerve usually resembles the peroneal, but occasionally specimens are more like the saphenous.

The question arises as to why two such contrasting pictures are to be found in different nerves. A clue to an explanation was found in the observation that when the form of the after-potentials of a nerve was not profoundly changed by asphyxia it was also not greatly altered by increased alkalinity. In a previous paper (1937b) it was shown that resistance to increased alkalinity could be brought about by providing a rich supply of calcium. If the concentration of ionized calcium is also to be the basis of the differential behavior during asphyxiation, it should follow that nerves of the saphenous type should resemble the peroneal type if they are made alkaline or deprived of calcium, and that nerves of the peroneal type should resemble the saphenous type if they are made acid or have their calcium content enriched. Experiments were performed in which the transformation was essayed and the results were in accord with the hypothesis.

The saphenous nerve, if asphyxiated at pH 7.85 instead of at pH 7.4, developed good positive after-potentials and spontaneous firing (fig. 6;

compare with fig. 5); and the peroneal nerve, if bathed for 3 hours in Krebs' solution without KCl but containing 5 times the normal amount of $CaCl_2$, showed less change in the positive potential and no firing (fig. 4, c and d). In the latter the high calcium content antagonized the effects of asphyxia just as it did the effects of a high pH (compare fig. 4 b with fig. 2 d for the differential response of the nerve at pH 8.0). For some reason not under-

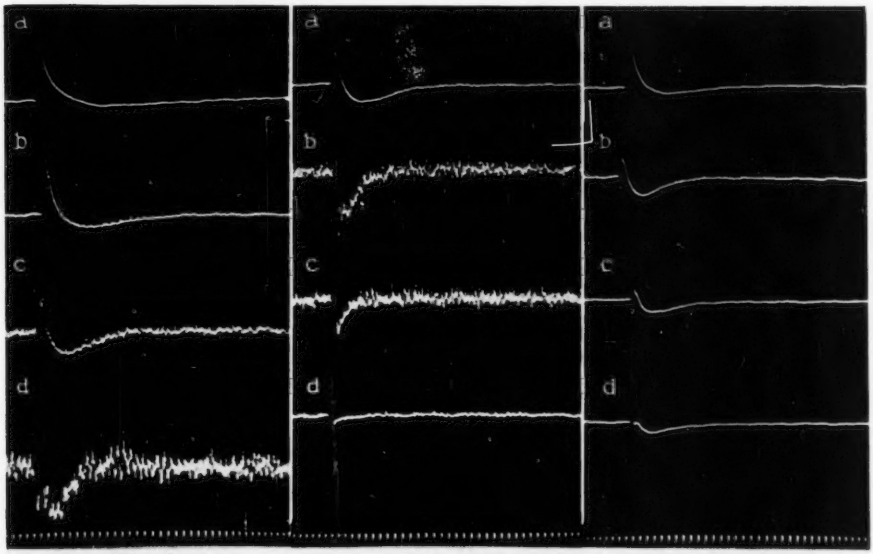

Fig. 2 Fig. 3 Fig. 4

Fig. 2. After-potentials at different pH values in a peroneal nerve (cat) at 37° C. *a*, at pH 7.4; *b*, at 7.6; *c*, at 7.8; and *d*, at 8.0.

Fig. 3. After-potentials in the same nerve as in figure 2 during asphyxia. *a*, after 2 minutes of N_2; *b*, after 5 minutes of N_2; *c*, after 8 minutes of N_2; *d*, after 13 minutes of N_2.

Fig. 4a and b. After-potentials in a saphenous nerve (cat) at pH 7.4, *a*, and pH 8.0, *b*.

Fig. 4c and d. After-potentials in the same nerve after 4 minutes of asphyxia *c*, and after 8 minutes, *d*.

stood, some nerves behave as though they possessed a greater supply of available calcium than other nerves.

Recovery from asphyxiation. Both the spikes and the after-potentials recovered promptly, the spike reaching normal size in 5 to 10 minutes. In analogy with the observations on frog nerve of Amberson, Parpart and Sanders (1931) and others, the negative after-potential initially far overshot its normal magnitude. During the first 5 to 10 minutes, the time depend-

ing upon the duration of the asphyxiation and other variables, the negative after-potential increased in height and duration. During this period it often had a well marked rising phase (fig. 7 a) as it does in frog nerve (Gasser and Graham, 1932). In the subsequent period the rising phase shortened, the initial part of the potential became higher and the duration

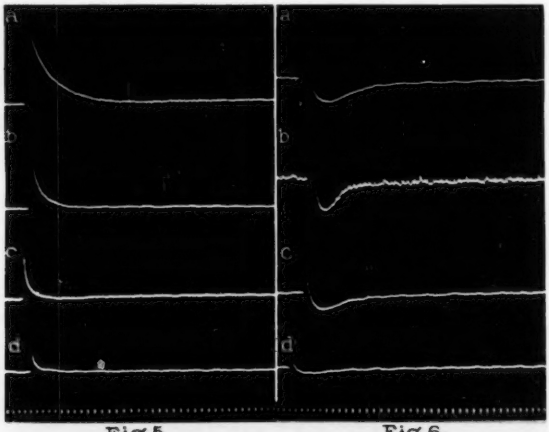

Fig. 5

Fig. 6

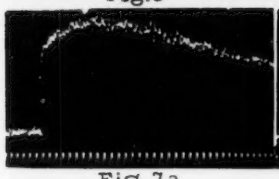

Fig. 7a

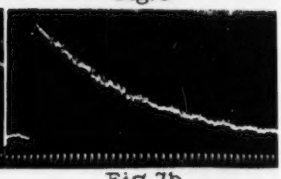

Fig. 7b

Fig. 5. After-potentials in a saphenous nerve during asphyxia. *a*, before N_2; *b*, after 2 minutes of N_2; *c*, after 4 minutes of N_2; *d*, after 10 minutes of N_2. Extreme case with no development of positive after-potential.

Fig. 6. After-potentials in a saphenous nerve asphyxiated at pH 7.85. *a*, before N_2; *b*, after 2 minutes of N_2; *c*, after 8 minutes of N_2; *d*, after 10 minutes of N_2. Large positive after-potential and spontaneous discharge are developed as in the peroneal nerve.

Fig. 7. After-potentials in a peroneal nerve, *a*, after 2 minutes' recovery in O_2 following asphyxiation, showing the rising phase in the after-potentials; *b*, after 10 minutes of O_2.

decreased (fig. 7 b). As this happened, the positive after-potential returned to its normal size and position. The time for complete recovery was variable and in general faster for the peroneal nerve than for the saphenous. In some nerves the end result was a permanent increase of potential with a normal configuration.

Mechanism of production of the increased negative after-potential during recovery. Gerard (1927) and Fenn (1930) have shown that the period following asphyxiation of frog nerve is marked by increased utilization of oxygen for hours and it is natural to suppose that the behavior of the nerve is associated with this altered metabolism.

Accumulation of catabolites was first considered. Lactate is known to accumulate in nerve during asphyxiation (Gerard and Meyerhof, 1927) and accordingly the effect of added lactate on the after-potentials was tried. As a result it was found that concentrations of *d*-lactate (neutral) up to 250 mgm. per cent were without effect upon the character of the potentials. Other catabolites may have an effect; but, because of the uncertainty about which ones to try, no further experiments of this type were performed.

Because of the parallelism which exists between them, hydrogen ions and calcium ions must be considered together. During the first 8 minutes of asphyxiation an alkaline reaction or deionization of the calcium would account for the changes in threshold and form of the after-potential, which appear during that period; and during recovery an acid reaction or an abundance of calcium ions would account for the large negative potential. It might be argued that lactic acid accumulates during the extinction period, changing the reaction to acid (phenol red injected under the nerve sheath did not change in color, but indicators in the tissue spaces would not give a satisfactory answer to the question). But it could with equal propriety be argued that the nerve had become alkaline. The nerve behaves as though it were alkaline in the early period of asphyxia and this postulated alkalinity might persist into the later stages. The overshooting of the negative after-potential on oxygenation would then be in keeping with the overshooting which occurs when a nerve is returned to pH 7.4 after having been held at pH 8.0 (Lehmann, 1937a). With this comparison, however, we are brought only a little closer to the solution of the problem, as up to the present time the latter phenomenon itself has received no explanation. It is quite possible that the postasphyxial developments may depend upon some mechanism which operates also after high alkalinity, for example, a transient overproduction of calcium ions.

If the concentration of calcium ions is to be the explanation of the behavior of the after-potentials during recovery from asphyxia and during restoration to a normal reaction after a period of alkalinity, the behavior of the nerve otherwise should be in accord with a concentration of calcium equivalent to that with which the after-potentials would correspond. In the absence of direct chemical tests only indirect evidence is available.

It is well known that calcium ions cause a rise in the threshold of excitation. If the large negative after-potentials are attributable to high calcium, the resting thresholds should be elevated and the curve of thresh-

olds should parallel the ability to produce negative after-potential. In accord with this argument, the thresholds were measured in the two conditions, the method used being the determination of the strength of shock which would produce a constant height of response. The results are shown in figure 1 a.

In the curve showing the course followed when a nerve is restored to pH 7.4 after having been kept at pH 8.0, the thresholds are seen to rise from the low values obtaining at the latter reaction to a height 70 per cent above normal, and then to fall again to normal over a period of 38 minutes. This period has the same duration as that in which the negative after-potentials have an augmented magnitude (fig. 1 b).

In the curve showing the course followed by the excitability when oxygen is readmitted to an asphyxiated nerve there is likewise a period of high thresholds (fig. 1 a). When excitability begins to return, the threshold first drops sharply ove ra period of 1 to 2 minutes. It then undergoes a marked secondary rise, and reaches normal only after 40 minutes have elapsed. This period again corresponds to the period of increased after-potentials (fig. 1 b).

Thus the idea of the operation of a similar mechanism after periods of asphyxia and periods of alkalinity receives support. The data are compatible with the postulate that the calcium is high; but the possibility of the existence of other factors acting like high calcium is not excluded.

Discussion. All the variations in the behavior of the nerve during asphyxia and recovery have been found to be consistent with the hypothesis that the state of the calcium concomitantly undergoes a series of changes. This fact brings up the question of whether the calcium activity is determined by the intervention of an altered hydrogen ion concentration, or whether it is responsible to some other mechanism. The low thresholds, the spontaneous discharges, and the after-potential configuration, found in the early period of asphyxia, are all characteristic of alkaline nerves. And the large negative after-potential and high thresholds, found in the early period of recovery, are characteristic of acid nerves or nerves recently restored to normal pH after a temporary period of alkalinity. The direction of the changes is such as to permit the hypothesis either that the nerve becomes alkaline, or that it becomes alkaline at first and later, during the period of extinction of the response, becomes acid because of the formation of lactic acid. But the magnitude of the changes is against this hypothesis. The behavior of the nerve in early asphyxia is like that at pH 8.0, a degree of alkalinity which the nerve could not be expected to reach; and the size of the negative after-potential during early recovery is much larger than can be imitated by making a nerve acid.

The greatest accumulations of catabolites during asphyxia which have been reported in the papers of Holmes and Gerard (1929) would account

for only very small shifts of pH. Certainly no changes could occur equivalent to the ones necessary for the imitation of the behavior of asphyxiated nerve. The deionization must have another cause, possibly liberation of an ion by which calcium is deionized or with which it forms a precipitate,—as the phosphate ion. Liberation of phosphate or a similarly acting ion in early asphyxia would account for the phenomena which occur at that time. As asphyxia proceeds it can readily be supposed that precipitation of the normally ionized calcium would upset an equilibrium with an unionized fraction leading to a mobilization of calcium ions in order to restore the original balance. The mobilized calcium in turn would be precipitated and as a result inactive calcium salt would accumulate. Then, when the deionizing factor is removed during the oxidative metabolism permitted by the reintroduction of oxygen, ionizable calcium would be liberated in concentrations in excess of normal and would remain in excess until the normal distribution of calcium is restored. This excess of calcium would account for the events in the transitional stage of recovery.

A similar process would occur when the calcium is deionized at a high pH; and it would be for that reason that recovery from the latter and recovery from asphyxia are brought into relationship.

When interpreted on the basis of Ca^{++} activity, the curve of thresholds obtained during asphyxia and recovery would have to be considered as made up of two parts. One part would be secondary to the state of the calcium and would be made up of the period of low thresholds during asphyxia (fig. 1a) and of the period of high thresholds during recovery. The other part would be attributable to another factor, possibly to the inactivation of an enzyme, and would account for the rise of threshold which occurs after the first few minutes of asphyxia. When oxygen is readmitted the enzyme factor would be reactivated and the threshold would drop to the level determined by the ionic balance. In one preparation the excitability almost reached the low level which developed in early asphyxia.

SUMMARY

The effect of asphyxia on excised peroneal, phrenic and saphenous nerves of the cat was investigated under controlled conditions of hydrogen ion concentration. In many respects the changes produced resembled those previously known to be true for frog nerve.

The after-potentials disappeared before the spike, 10 minutes on the average being required for the former, as compared with 30 minutes for the latter. They also returned more slowly to normal, taking 50 minutes in comparison with 8 minutes for the spike.

During the first 6 to 8 minutes of asphyxiation the threshold of excitation fell markedly. Then it rose abruptly to the point of complete inex-

citability. On readmission of oxygen the threshold again fell rapidly to near the normal level; it then rose for the second time and remained high for about 50 minutes. This secondary rise resembles the curve of thresholds obtaining when an alkaline nerve is restored to normal.

The changes in the threshold are interpretable on the basis of an alteration of the ion balance, and all the other happenings are consistent with this interpretation. During the period of low thresholds the after-potentials are decreased and the nerve becomes spontaneously active as it does in a state of low calcium. During the time of high thresholds in the recovery period, the negative after-potential is augmented as it is in nerves treated with calcium and in nerves recently restored to normal from an alkaline reaction.

The peroneal and saphenous nerves differ characteristically with respect to their behavior during asphyxia. Other nerves resemble one or the other type to a greater or less degree.

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EFFECTS OF ANDROGENIC SUBSTANCES IN HYPOPHYSECTOMIZED RATS

EUGENE CUTULY, D. ROY McCULLAGH AND ELIZABETH C. CUTULY

From the Department of Biochemical Research, Cleveland Clinic, Cleveland, Ohio

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Walsh, Cuyler and McCullagh (1) studied the effect of androtin (fat soluble extract of male urine) on adult hypophysectomized male rats and found that it prevented atrophy not only of the accessory organs but also of the testes. These observations have recently been confirmed by Nelson and Gallagher (2). However, these latter investigators, using urinary male hormone, and Vatna (3) using bull-testis extracts, were unable to detect any repair of the testicular tubules, if injections were begun three weeks after hypophysectomy. In the light of recent investigations (4, 5) it seems fairly certain that the urinary extracts employed by Walsh et al. were rich in androsterone, while those employed by Vatna were rich in testosterone. Since both these substances are now available in crystalline form, it seemed advisable to determine the effects of these and other synthetic androgens[1] in hypophysectomized rats. In all we have studied eight such substances. At present we wish to report only the results obtained with androsterone, testosterone, and dihydroandrosterone benzoate.

MATERIALS AND METHODS. Adult male rats were used in this study. Table 1 shows the dosages of the androgenic substances employed, the number of animals in the experimental and control groups, and the average absolute and relative weights of the sexual organs of the animals. It will be noticed that for every hypophysectomized treated group, with the exception of group VII, there are separate hypophysectomized control and normal control groups. Groups which are so classed together constitute distinct series. Animals in each series were uniform as to size, age and strain, and in some instances were littermates; but the animals in the different series were not of uniform stock. Comparison of absolute weights, therefore, is permissible within each series, but not among different series. In order to make inter-series comparisons possible, relative values were computed with normal control values considered as 100.

As the result of preliminary experiments indicated that the intraperi-

[1] Supplied through the courtesy of Dr. Erwin Schwenk, Schering Corp., Bloomfield, N. J.

toneal administration of androgenic substances produced indefinite and irregular results, all injections were made subcutaneously. Injections in hypophysectomized animals were begun one day after operation and continued daily for 18 days. The animals were autopsied one day after the final injection. The material to be injected was dissolved in sesame oil; the volume of each injection was uniformly 0.5 cc., except in group IX where it was 0.6 cc.

TABLE 1

Average absolute weights (grams) and relative weights (normal control values = 100) of sexual organs of hypophysectomized rats injected subcutaneously daily for 18 days

GROUP	NUMBER OF RATS	TESTES		SEMINAL VESICLES		VENTRAL PROSTATE		DORSAL PROSTATE	
		Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
Dihydroandrosterone benzoate (1.25 mgm. daily)									
I. Hypophysectomized treated	7	0.48	20	0.214	38	0.105	46	0.072	52
II. Hypophysectomized control	3	0.45	19	0.046	8	0.017	8	0.023	17
III. Normal control	4	2.42	100	0.558	100	0.227	100	0.137	100
Androsterone (1.50 mgm. daily)									
IV. Hypophysectomized treated	7	1.57	65	0.431	58	0.239	74	0.139	75
V. Hypophysectomized control	4	0.54	22	0.096	12	0.034	10	0.042	23
VI. Normal control	8	2.41	100	0.745	100	0.325	100	0.185	100
Testosterone (0.45 mgm. daily)									
VII. Hypophysectomized treated	3	1.09	36	0.473	73	0.127	56	0.103	78
VIII. Normal control	2	2.73	100	0.651	100	0.226	100	0.133	100
Testosterone (1.50 mgm. daily)									
IX. Hypophysectomized treated	3	1.37	59	0.906	136	0.251	76	0.144	97
X. Hypophysectomized control	1	0.56	24	0.065	10	0.025	8	0.038	26
XI. Normal control	2	2.32	100	0.668	100	0.331	100	0.148	100

All organs were weighed in the fresh state and then fixed in Bouin's or Zenker's fluid. Paraffin sections of the tissues were cut at 5 micra and stained with hematoxylin-eosin, iron hematoxylin-light green, or with Mallory's triple stain.

Serial sections of the pituitary fossae of all treated animals (except for a few in group I) were carefully examined microscopically. No remnants of anterior lobe were discovered in any instance.

RESULTS. 1. *Scrotum.* It has been known for some time (6) that

following hypophysectomy in the rat, the scrotum gradually regresses and the testes come to occupy an inguinal or abdominal position. In the hypophysectomized rats injected with androsterone and with the 1.5 mgm. dose of testosterone, the scrota did not regress but remained pendent and vascular, and the testes remained scrotal in position. Androsterone seemed to cause greater turgescence and flushing of the scrotum than testosterone. The smaller dose of testosterone seemed only to retard scrotal atrophy. Dihydroandrosterone benzoate produced only a very slight scrotal response in hypophysectomized rats as judged by the degree of flushing and turgescence; the testes of these animals were inguinal in position at the time of autopsy.

2. *Testes.* The values in table 1 show that 1.5 mgm. of androsterone largely prevented the testicular atrophy which invariably follows hypophysectomy. Equivalent doses of testosterone (IX) were slightly less effective than androsterone, and smaller doses (VII) were considerably less effective. Dihydroandrosterone benzoate completely failed to arrest testicular shrinkage.

a. *Interstitial cells.* Histologically the testes of all the hypophysectomized rats treated with the androgenic substances showed marked atrophy of the interstitial cells and this was similar in every way to that which occurred in the testes of control hypophysectomized rats. It seems worthy of note that two types of interstitial cell degeneration were observed. The usual type was characterized by an apparent decrease in the size of the nucleus, which seemed to stain more intensely, and a profound reduction in the amount of cytoplasm. A more unusual type of degeneration was seen in interstitial cells in whose cytoplasm relatively large masses of yellowish pigmented granules were prominent. At times this pigment was so abundant that the nucleus, which was usually pyknotic, was displaced to the periphery of the cell. Similar pigmentary degeneration was observed to occur in the reticular zone of the adrenal glands of these animals. These pigmentary changes are similar to those described by Rasmussen (9) as occurring in the testes of the woodchuck in the autumn and during hibernation and to those described by Zalesky (10) as occurring in the adrenals of castrated guinea pigs.

b. *Tubules.* The histological appearance of the tubules of the testes in the injected hypophysectomized rats varied, depending on the substance administered and the dose. Androsterone and testosterone in daily doses of 1.5 mgm. apparently caused complete maintenance of most of the testicular tubules; the latter contained an intact and structurally normal seminiferous epithelium. Structurally normal spermatozoa were present in abundance also in the epididymides. Varying degrees of tubular damage, however, were discernible in all the testes. The testes of the hypophysectomized rats injected with 0.45 mgm. of testosterone contained more degenerated tubules than did those treated with the larger dose, and

tubular maintenance, when it did occur, was not as complete as in the other group. In a few instances the tubules of the testes of the animals treated with dihydroandrosterone were not so profoundly atrophic as in untreated controls, but such findings were not sufficiently marked nor consistent to be considered significant.

c. *Accessory sexual organs.* Dihydroandrosterone was least effective in maintaining the accessories. Testosterone at both dosage levels produced relatively heavier seminal vesicles than did androsterone. However, the relative weights of the ventral prostates of the animals injected with 1.5 mgm. of androsterone were greater than those in animals injected with 0.45 mgm. of testosterone. The dorsal prostates of androsterone-treated rats were relatively as heavy as those of rats treated with 0.45 mgm. of testosterone, but appreciably smaller than those of rats treated with the larger dose of testosterone.

While the histological appearance of the accessory sexual organs of all treated rats could be characterized as essentially normal, certain differential effects are worthy of note. For example, when androsterone and testosterone were administered in equivalent amounts (1.5 mgm.) the ventral prostates were maintained to practically the same degree—74 per cent and 76 per cent normal, respectively. The epithelial response of the ventral prostates of the androsterone-treated animals, however, was structurally inferior to that in the testosterone-treated rats: in the former group the prostatic epithelium was comparatively low while in the latter group the epithelium was very tall. The prostates of testosterone-treated animals, on the other hand, contained very little secretion in the acini, while those of the androsterone-treated rats contained a considerable amount of secretion. Both the epithelial response and the amount of secretion in the seminal vesicles of testosterone-treated animals, however, were greater than in androsterone-treated rats. In the two testosterone-treated groups (VII and IX) the marked difference in relative weights between the heavier dorsal prostates and the lighter ventral prostates (in each case the difference being approximately 20 per cent) could be attributed entirely to the presence of secretion in the dorsal portions of the glands and to its absence in the ventral portions.

Discussion. The fact that the scrota of hypophysectomized animals do not regress when the animals are injected with androgenic substances has already been interpreted by us (7) as indicating that the scrotum is under the control of the male sex hormone. Recently Hamilton (8) has independently expressed the same idea.

The maintenance of testicular tubules in rats by androgenic substances in the absence of hypophyseal hormones leads one to question whether such an effect is the result of a direct stimulating action of male hormone on the seminiferous epithelium or whether it is brought about indirectly. In the light of Moore's studies (11) which have shown that proper scrotal

function is indispensable to spermatogenesis in the rat and other mammals, it seems obvious that the scrotal atrophy which invariably occurs following hypophysectomy in the rat would in itself be sufficient to cause tubular injury. It seems quite possible, therefore, that the administration of androgenic substances to hypophysectomized rats prevents scrotal atrophy and thus provides the tubules with an environment which is suitable for and essential to the continuance of gametogenesis. This idea is supported by the fact that the degree of tubular maintenance in our animals seemed to be correlated nicely with the degree of scrotal maintenance. Tubular maintenance in such a case would be only indirectly effected by male hormone. If this is correct, it would seem that spermatogenesis, to a certain extent, is independent of any direct recognized hormonal stimulation. To how great an extent spermatogenesis in the rat is dependent only on environment and independent of direct anterior pituitary stimulation remains to be determined.

Nelson and Gallagher (2) have demonstrated that spermatogenesis in hypophysectomized rats could not be revived by urinary extracts of male hormone if the injections were begun after involution of the testis tubules had occurred. Similar results have been obtained by us in rats hypophysectomized previously and injected with testosterone or androstenediol, despite the fact that in some of our animals the scrota were restored to an apparently normal condition and the testes redescended in them (unpublished experiments). While such results strongly indicate that androgenic substances are, unlike anterior pituitary substances, incapable of repairing the tubular damage caused by hypophysectomy, it should be borne in mind that the type and dose of androgenic substance employed and the duration of the injections are important factors which should be investigated further in this respect.

The idea that spermatogenesis in the rat is to some extent, at least, independent of any direct hormonal stimulation has already been advanced by us (7). We interpret the recent findings of Hamilton (8) and of other investigators as strong supporting evidence of this view. Nelson (12) has found that progesterone can cause tubular maintenance in hypophysectomized rats. Fevold et al. (13) have shown that yeast extracts also are capable of preventing atrophy of the testis tubules in hypophysectomized rats. Furthermore, the latter workers mentioned the fact that the yeast extracts prevented scrotal atrophy in their animals.

The important rôle played by the scrotum has been so emphasized by this study that it seems wise to call attention to another point concerning gonadokinetic substances. Reports by Smith et al. (14) and by Evans et al. (15) have indicated that the germinal epithelium of the testes of hypophysectomized rats can be selectively stimulated without any obvious morphological or physiological effect on the interstitial cells. Neither of these groups of investigators mentioned the condition of the scrota of

their animals or the position of the testes. If the scrota of their animals were functioning properly, it may be questioned as to just what extent the observed gametokinetic effects could be attributed to a selective gametokinetic principle, for an intact scrotum would imply that male hormone was being produced. At present, it is believed that the production of male hormone is stimulated by the "luteinizing" or interstial cell-stimulating principle of the anterior lobe of the hypophysis. If the latter substance were present, it is possible that sufficient male hormone was being produced by the testes to maintain the scrotum, but not enough to affect the other accessory sexual organs. Were such the case, the interpretation of the results obtained by these investigators would have to be modified.

CONCLUSIONS AND SUMMARY

1. Dihydroandrosterone, androsterone, and testosterone were injected into hypophysectomized rats for 18 days. All three substances at the dose levels employed caused varying degrees of maintenance of the accessory sexual organs, but only androsterone and testosterone prevented to a large extent atrophy of the testis tubules. Degeneration of the interstitial cells was not arrested by any of these substances.

2. Evidence is adduced that: *a*, scrotal function is maintained by the male sex hormone; *b*, proper scrotal function is indispensable to spermatogenesis; *c*, spermatogenesis in the rat is to a certain extent independent of any direct recognized hormonal stimulation.

3. The results of this investigation indicate the need of caution in the interpretation of apparently purely gametokinetic effects in the male rat.

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URINE FORMATION UNDER ANOXIA IN DOGS[1]

L. A. TOTH

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

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In experiments on the frog Adolph (1934, 1935) found that low oxygen tensions inhibited urine production. The question arises: do mammals react similarly to shortage of oxygen? No satisfactory information could be found in the literature on the effect of anoxia alone on the rate of urine production in mammals, although variations in urine volume have been mentioned in reports primarily concerned with the effects of low oxygen and of respiratory obstruction on the general metabolism and blood composition. Scattered data also exist concerning variations in urine volume at high altitudes, during the reduction of barometric pressure in decompression chambers, and during the administration of cyanide.

In the present work, two series of experiments were carried out, one on anesthetized sacrifice dogs and the other on dogs with urinary bladder fistulas.

ANESTHETIZED DOGS. In the first series, the dogs were given diallylbarbituric acid plus urethane (Dial[2]) by stomach tube. After complete anesthesia, one of the carotid arteries was cannulated for blood pressure recording by a mercury manometer; the trachea was cannulated for the administration of the low oxygen; and the ureters, exposed through a ventral incision in the abdominal wall, were cannulated a short distance from the bladder.

The oxygen mixtures were made up before each experiment in two large spirometers of 500- and 300-liter capacities. The percentages of oxygen and carbon dioxide were determined by gas analysis. Carbon dioxide was not added in the later experiments after it was found that in proportions up to 4 per cent it did not alter the urinary responses of the animals to anoxia. The gas mixtures were administered to the dogs by connecting the spirometer to the cannulated trachea by wide-bore rubber tubing and flutter valves so that the animal breathed from the spirometer and out into the room air.

[1] A preliminary report of this work appeared in the Proceedings of the American Physiological Society, This Journal **113:** 131, 1935.

[2] Dr. C. C. Haskell, of the Ciba Company, New York City, generously supplied the anesthetic.

The urinary rates were determined by collecting urine samples separately from the cannulated ureters over 15- or 20-minute periods; but in some of the experiments the drops of urine were counted in 2-minute periods. In all of the experiments, control urinary rates were determined after the animal had been prepared and while it was breathing room air through the flutter valves.

The dogs were last fed 16 hours before the experiment, but they had access to water until the administration of the anesthetic. No diuretic was used in any of the experiments.

The chloride concentration of the urine was determined on 0.1 cc. by the modified Volhard-Harvey method (Peters and Van Slyke, 1932a). Urea plus ammonia was also determined on 0.1 cc. by the Van Slyke and Cullen urease-aeration method (Peters and Van Slyke, 1932b). Qualitative sugar tests using Benedict's solution, and protein tests with acetic acid plus heat, were made on 1 drop of urine.

Water excretion. Although at times polyuria resulted from the breathing of low-oxygen mixtures, the general response of the dog was oliguria of varying degree. The response of dog j (fig. 1) can be considered as usual; with every period of anoxia, the urinary rate was diminished. The renal response of the animal to anoxia may not be an immediate oliguria but with the continued breathing of the oxygen-poor mixture, the polyuria which occasionally resulted, for example in dog q (fig. 1), gradually gave way to an oliguria. The readministration of room air generally produced polyuria which lasted for varying lengths of time.

The arterial blood pressure of the animals during the anoxia rose and remained elevated during the period. Even though the blood pressure increased, the urinary rate decreased (dog j, fig. 1); hence the general systemic blood pressure did not induce the oliguria.

That the renal responses cannot be attributed entirely to the low oxygen per se is illustrated by the responses of dog w (fig. 2). Anoxia lasted only 3 minutes, but polyuria lasted 1 hour.

Among the responses that occur in mammals as a result of reducing the oxygen supply is an increased secretion of epinephrine. The evidences for this are given by Cannon (1929) and by Sato, Inaba and Takahashi (1932). To determine whether the increased activity of the adrenals as a result of anoxia was a factor contributing to the urinary response, an experiment was conducted in which the procedure was the same as above, except that in addition the lumbo-adrenal veins were occluded by ligatures. After the urinary response to anoxia was observed, the veins were released, a new urinary control rate determined, and low oxygen was again administered. Without the adrenals the response was polyuria; with the adrenals, the response was possibly oliguria (fig. 3). Emphasis cannot be placed on one experiment, but evidently the adrenal glands play an important part in the renal response to low oxygen.

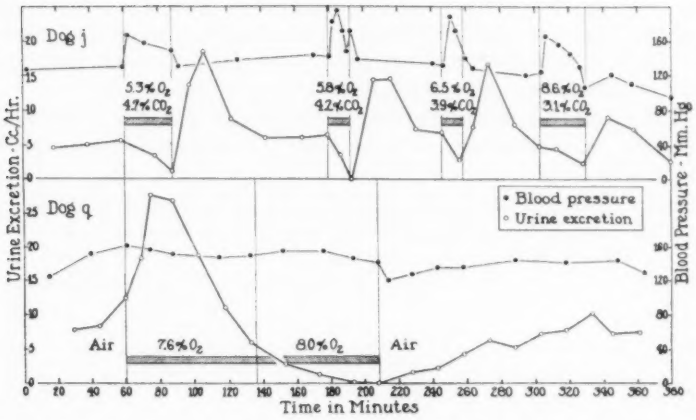

Fig. 1. Effects of anoxia on urine excretion and blood pressure in anesthetized dogs.

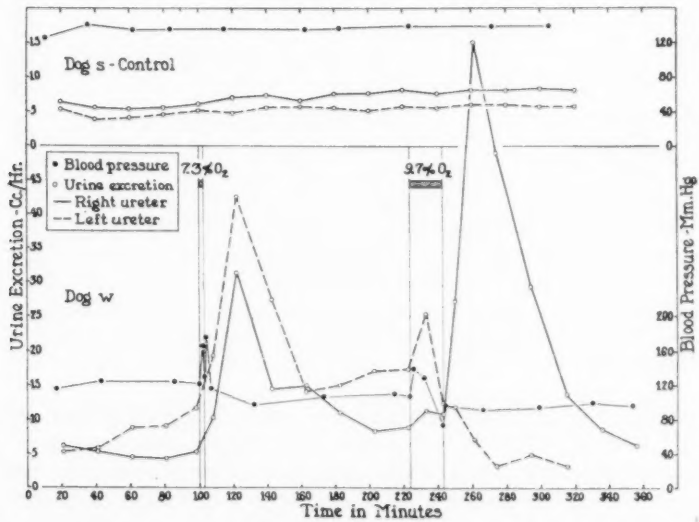

Fig. 2. Effects of normal, and of low oxygen tensions, on urine excretion and blood pressure in anesthetized dogs.

Dog w (fig. 2) illustrated the fact, observed occasionally, that both kidneys do not necessarily show the same response; during the second low-oxygen period, the left kidney manifested greater polyuria than the

right. In the recovery period, the left kidney showed a diminution in excretory rate; the right, a tremendous polyuria. Generally speaking, however, both kidneys reacted similarly, and for this reason most of the graphs presented have the excretory rate for only one kidney.

Full-time control experiments were conducted in order to determine whether the surgical procedure carried out in the preparation of the animals could alone give a renal response (dog s, fig. 2); the dog was breathing room air through the flutter valves during the entire experiment. The urinary rates and the carotid blood pressure were constant.

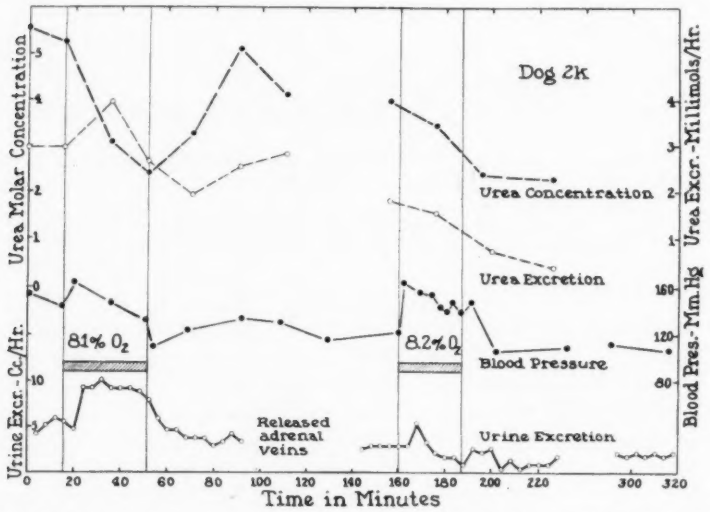

Fig. 3. Effects of anoxia with and without occlusion of adrenal veins on urine excretion, blood pressure, urea concentration, and rate of urea excretion in an anesthetized dog.

A summary of the experiments carried out on anesthetized animals is given in table 1. The onset time for each response was determined by examining the curve of the urinary rate and noting the urinary period in which the curve showed a definite change. The time from the beginning of the anoxia to the midpoint of this period was taken as the onset time. The time of the recovery of rate of urine excretion while breathing room air was determined in this same way.

The experiments of table 1 are divided into two groups, those experiments which resulted in oliguria during the anoxia, and those which resulted in polyuria. No percentage of low oxygen can be taken as an invariable limit for inducing oliguria or polyuria. But the rapidity of

onset of the response is correlated with the oxygen percentage; the lower the oxygen percentage, the more quickly the kidney responds. Another

TABLE 1

Renal responses to anoxia in anesthetized dogs

DOG NUMBER	GAS COMPOSITION		DURATION OF ANOXIA	EXCRETION RATE		ONSET TIME	RECOVERY TIME	URINE RECOVERY IN AIR
	O_2	CO_2		Left ureter	Right ureter			
	per cent	*per cent*	*minutes*	*per cent**	*per cent**	*minutes*	*minutes*	*per cent**
e	9.0–7.8		68	35		13	100	95
j-4	8.6	3.1	27		50	17	8	130
2k	8.2		27		55†	20	10	80
2j	8.1–7.5		45		35	8	28	55
e-2	8.0	0.2	59	25		6	20	100
o	7.8		123	0	0	23	190	
q	7.6–8.0		149	25†	25†	82	100	110
p	7.6		95	30†	60†	66	130	95
z	7.5		24		50†	20	8	290
2c	7.3		74	50†	75†	50	10	90
l	6.8	4.2	31	10	40	7	8	70
j-3	6.5	3.9	12		40	5	4	145
k-2	6.4	3.9	31	45	25	11	70	135
e-1	6.3	4.8	49	30		28	5	120
d-2	6.2	0.2	27	30		6	5	105
j-2	5.8	4.2	13		35	4	6	180
j-1	5.3	4.7	28		50	10	5	230
g-2	5.0	4.7	20	25	15	5	7	95
k-1	4.8	4.1	23	40	30	13	45	175
w-2	9.7		19	110	130	5	4	570
d-1	8.5	2.0	67	135		7	5	175
2i	7.7		64	120		30	10	120
w-1	7.3		3			3		440
g-1	6.6	3.7	15	110	170	5	8	95
i	5.3	3.8	32	140	165	5	7	450
r	20.9 (control)			35‡	45‡			
s	20.9 (control)			130‡	140‡			

Where several experiments were performed on one dog they are indicated by a number after the letter which identifies the animal.

* Percentage of normal (control) rate. Urine recovery in air is the maximum rate which was attained, not necessarily at the "recovery time."

† Polyuria preceded oliguria during anoxia (see fig. 1).

‡ Changes were gradual and progressive. The urine rates during the first hour were considered as 100 per cent.

possible correlation is that the longer the duration, the longer the recovery time. These two correlations are especially noticeable in the oliguria experiments.

The oxygen saturation of the blood during all of the periods of anoxia was decidedly below the normal value, as judged from the color of the blood vessels on the urinary bladder wall. Oxygen analyses were carried out on arterial blood drawn during the period of anoxia in two experiments (dogs g and i); the blood during the low-oxygen period contained only 47 per cent of the normal content, and in the latter only 30 per cent.

The data given in table 1 have been supplemented by Van Liere and co-workers (1935), who reported that brief periods of anoxia, in barbitalized dogs, resulted in oliguria. The proportion of their experiments that resulted in polyuria, one in fifty experiments, is less.

Solute concentrations and excretions. The changes in the urinary urea plus ammonia concentration in urine as a result of the anoxia are illustrated in figure 3. The concentration during the first anoxial period decreased with the increase in the urinary rate. Although the adrenal veins had apparently been occluded, the variation in the urea concentration was the same during the polyuria whether epinephrine had access to the circulation or not. During the second anoxial period, the concentration again decreased, but in this case the urinary water rate also decreased. The rate of urea excretion varied directly as the water excretion rate.

The chloride concentration of the urine, after complete anesthesia, was extremely low in all the experiments. Probably the anesthetic, or the increased respiratory rate which resulted as the anesthetic was taking effect, influenced the urinary chloride concentration. With the concentration already low, no significant effect of the anoxia on the concentration was observed.

A summary of the urinary urea analyses, and of the protein and sugar tests, from all the experiments, is given in table 2. Proteinuria appeared regularly during oliguria but never with polyuria. Glycosuria occurred in the majority of dogs during or shortly after the anoxial period in the polyurias and oligurias.

Bladder-fistula dogs. Although the above experiments seemed to show that oliguria was the usual result of anoxia, it was possible that the experimental surgical procedure and the anesthesia were contributing factors. To decide this point a series of experiments was performed on unanesthetized dogs with urinary bladder fistulas; experiments were repeated on the same dogs after they had been anesthetized.

After having recovered from the operation for the urinary bladder-fistula, which opened to the outside just anterior to the symphysis pubis, the dog was trained to remain quietly in a stall, suspended by wide cloth straps, and to breathe through an air-tight face mask and flutter-valves. During the experiment a glass cannula in the fistula insured complete drainage of the urine into a funnel and a small graduate which were strapped under the fistula. No ill consequences were suffered by the animals because of the operation or the experimental procedure.

The gas mixtures were prepared and analyzed as previously described in this report. The same chemical analyses and tests were carried out on the urine samples as those in the anesthetized dog experiments.

Water excretion. The urinary responses are shown in section A of figure 4. Experiments 9 and 14 resulted in polyuria, the time of onset of polyuria apparently being correlated with the severity of the anoxia. In a full-time control experiment (expt. 16), no significant variation of the excretory rate is observed. The presence of carbon dioxide in the inspired low oxygen mixtures appeared to prevent the polyuria in dog x (expts. 18 and 19, table 3), but not in dog 2g (expts. 7 and 8, table 3). Table 3 summarizes all of the experiments carried out on the three dogs with urinary bladder fistulas. The threshold oxygen percentage for polyuria appears to be between 11 and 10 per cent. The exceptions to this are experiment 2e-2, in which 7.4 per cent oxygen did not cause any significant change from the normal rate, and experiments x-18 and x-19, just mentioned.

TABLE 2

Summary of chemical analyses of urine from anesthetized anoxial dogs

	NUMBER OF EXPERIMENTS	UREA CONCENTRATION		PROTEIN		SUGAR	
		Decrease	No change	Increase	No change	Increase	No change
Oliguria	16	8	5	10	6	9	7
Polyuria	6	4	1	0	6	4	2

The figures indicate the number of experiments in which each result was found.

Since the fistula dogs responded to anoxia with polyuria, a result entirely different from the anesthetized operated dogs, three experiments were performed on the same animals under anesthesia. Dog x-20 (fig. 4) was lying on its back; the urine was siphoned into a graduate through a narrow rubber tube connected to a cannula in the fistula. Dogs x-17 and 2g-11 (table 3) were suspended, legs down. It appears that neither the anesthetic nor the body position is a factor in the production of polyuria in fistula dogs, for polyuria occurred in all three.

Solute concentrations and excretions. Anoxia in the bladder-fistula dogs affected the urinary urea (plus ammonia) concentration in the same way as it did in those sacrifice dogs that responded with a polyuria, but urinary chloride concentration was affected differently. With the onset of polyuria, the urea concentration fell; but the chloride concentration rose (fig. 5). As the polyuria wore off, especially in the recovery period, both concentrations gradually approached the control levels. When the inspired air contained carbon dioxide, the increase in the chloride concen-

tration of the urine was prevented; the urea concentration, however, still decreased. At no time did glycosuria occur as a result of the anoxia, and the urinary protein continued the same as it was in control periods. A summary of these analyses and tests on the fistula dogs is given in table 4.

Discussion. *Water excretion.* Possible factors bearing on the kidney responses to anoxia are: 1, tubular reabsorption; 2, the acid-base equilibrium of the blood; 3, the plasma concentration; 4, the sympathetic nervous system and the adrenal glands.

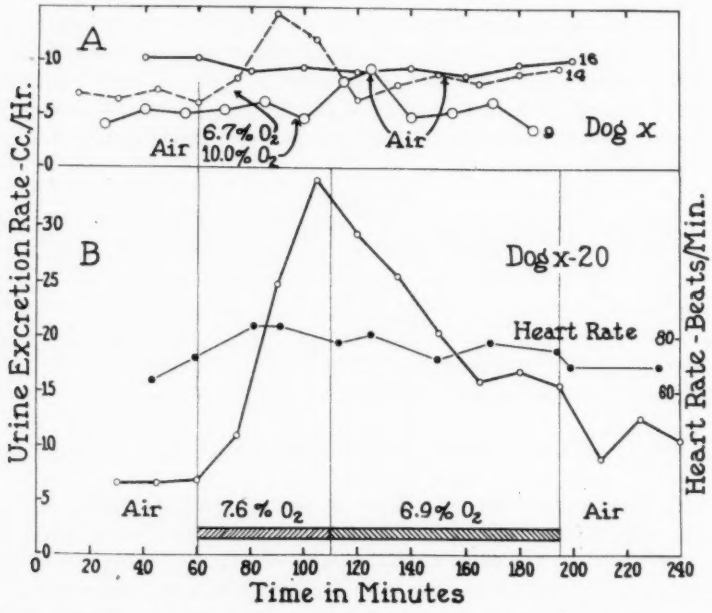

Fig. 4. Effects of anoxia on urine excretion and heart rate in anesthetized and unanesthetized urinary-bladder-fistula dogs.

If the anoxemia which results from the respiration of low oxygen asphyxiates the tubular epithelium of the kidney, the reabsorption of filtrate would be interfered with. Such an effect, which was said to be the basis for the response that Starling and Verney (1924) and Bayliss and Lundsgaard (1932) obtained by perfusing the kidney with cyanide, should have resulted mainly in polyuria and a urine approaching the plasma in composition. However, in the present work the sacrifice dogs generally showed oliguria, and the urinary urea concentration never fell nearly as low as the blood urea concentration.

If changes in the acid-base equilibrium of the blood, for instance the blowing off of carbon dioxide, were affecting the urinary rate, the presence of this gas in the inspired air should have altered the renal responses.

TABLE 3

Renal responses to anoxia in fistula dogs

DOG NUMBER	GAS COMPOSITION		DURATION OF ANOXIA	EXCRETION RATE	ONSET TIME	RECOVERY TIME	URINE RECOVERY IN AIR
	O_2	CO_2					
Unanesthetized animals							
	per cent	*per cent*	*minutes*	*per cent**	*minutes*	*minutes*	*per cent**
x-4	13.8		87	85	20	25	70
x-2	13.7		42	75	8	23	110
x-5	13.3		118	100		8	80
x-7	11.6		102	90	50	8	100
x-9	10.0		65	170	50	8	110
x-8	9.9		79	160	50	30	110
x-13	7.9		89	230	22	10	80
2e-1	7.8		31	130	20	8	105
2g-3	7.8		60	155	30	50	145
2g-4	7.6		70	150	22	20	75
x-19	7.5	4.3	51	120	20	8	110
2e-2	7.4		70	90	35		90
2e-3	7.4		90	130	30	20	140
2g-7	7.0	4.0	43	175	8	8	100
x-14	6.7–7.6		92	200	23		130
2g-8	6.6	3.6	57	135	8	20	115
2g-6	6.5		59	180	8	20	195
x-18	6.4	4.3	71	80	20	30	100
x-15	5.6–6.9		161	220	8		180
x-16	20.9 (control)			95			
2g-1	20.9 (control)			95			
Anesthetized animal							
x-20†	7.6–6.9		135	610–320	8	8	220
x-17‡	7.2		164	630–220	8	80	260
2g-11‡	6.5		25	170	5		

* Percentage of normal (control) rate. Urine recovery in air is the maximum rate which was attained, not necessarily at the "recovery time."

† Dog on its back.

‡ Dog suspended in stall.

This was not always the case. Again, changes in the acid-base equilibrium of the blood resulting from interference with the normal oxidation of organic acids, mainly lactic acid, might alter the normal renal function; but there is no evidence that they do.

The blood and plasma concentrations of solids remained fairly constant during the course of any one experiment; when any variation occurred, the changes were slight and progressive. This information was obtained from four anesthetized splenectomized dogs that were subjected to the same range of oxygen percentages and for the same lengths of time as those on which the urinary rates were determined. The analyses carried out on heparinized blood were: hematocrit and water content of whole blood, and refractive index and specific gravity of plasma. With this

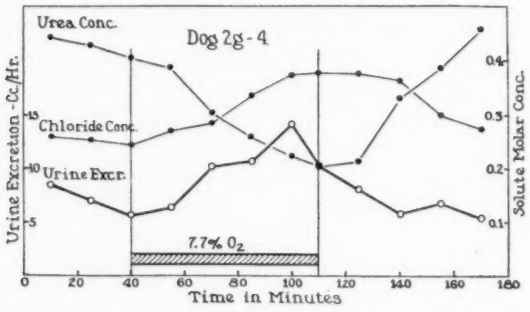

Fig. 5. Effects of anoxia on urea and chloride concentrations and water excretion in an unanesthetized urinary-bladder-fistula dog.

TABLE 4

Summary of chemical analyses of urine from fistula dogs

	NUMBER OF EXPERIMENTS	UREA CONCENTRATION		CHLORIDE CONCENTRATION		PROTEIN	
		Decrease	No change	Increase	No change	Increase	No change
Unanesthetized	13	9	0	6	3*	3	9
Anesthetized	3	2	1	1	1	0	2

The figures indicate the number of experiments in which each result was found.
* Low oxygen mixtures contained carbon dioxide.

constancy the variations in the water excretion rate can hardly be attributed to blood dilution or concentration. That small changes in the blood concentration may occur during anoxia resulting from breathing low oxygen in morphinized dogs is also indicated by data reported by Kasugai (1935), who found an increase in the serum protein concentration with a decrease in the colloidal osmotic pressure in dogs breathing 10 per cent oxygen. Such a fall in the colloid osmotic pressure might result in polyuria.

The anoxia may affect the kidneys through the sympathetic nervous

system (as suggested by Van Liere and co-workers, 1935). However, if this were the major factor affecting the kidney, no variation in the urinary rate should occur whether the epinephrine was entering the general circulation or not (dog 2k). The effect of different rates of administration of epinephrine on urinary excretion is reported in the following paper. The renal response of the urinary bladder-fistula dogs to low oxygen was polyuria, and under no conditions present in this series of experiments was a significant oliguria observed. It is possible that the secretion of epinephrine in the anesthetized dog is greater than in the unanesthetized animal. Kodama (1924) reported that the highest rate of epinephrine secretion in the anesthetized dog in response to complete asphyxia for 2 minutes was 7.38 micrograms/kgm./min., while Sato, Inaba, and Takahashi (1932) reported 1.6 micrograms/kgm./min. as the highest figure that they observed in the unanesthetized dog.

When the bladder-fistula dogs were anesthetized, anoxia still produced polyuria. A difference in the anesthetized fistula dog and the sacrifice dog is the surgical manipulation (cannulation of the trachea, carotid artery, and ureters) which the sacrifice animals underwent in their preparation for the experiment. The surgical manipulation may have changed the conditions existing in the animals, so that, for instance, like or unlike increased secretion of epinephrine acted differently in the sacrifice and bladder-fistula dogs.

Solute concentrations and excretions. Anoxia may affect the chemical composition of the urine by affecting the tubular epithelium, the glomerular membrane, or the blood pressure of the glomeruli. It may affect all three factors to varying degrees; in such a case, analysis of the responses of the constituents of the urine becomes extremely involved.

During the oliguria resulting from the anoxia in the sacrifice dogs the urea concentration decreased (fig. 3), but since the water excretion also decreased, the change in the urea concentration cannot be attributed to the increased excretion of water. This explanation might, however, apply to the fall in urea concentration which resulted during the polyuria in the sacrifice animals, and almost always in the polyuria occurring in the bladder-fistula dogs (fig. 5). As the oliguria or polyuria diminished, the urea concentration gradually approached the control level.

The urea excretion rate usually varied as the water excretion in both the sacrifice dogs and the urinary bladder-fistula dogs.

Van Slyke and co-workers (1934) report that the urea clearance parallels the blood flow of the kidney in the dog. With a decreased blood flow, the urea clearance was probably decreased. The decreased blood flow might be attributed to the increased epinephrine concentration of the blood which occurs during the anoxia, the epinephrine exerting a specific influence on the afferent and efferent arterioles of the glomeruli. This point will be discussed in greater detail in the following paper.

The urinary chloride concentration of the bladder-fistula dogs increased as the polyuria occurred. This effect on the chloride concentration appears to be a separable one, for the presence of carbon dioxide in the inspired air prevented this increase. These changes in the chloride and urea concentrations are unlike those in experiments concerning the effects of cyanide on renal function.

The chloride excretion rate in the fistula dogs increased during anoxia, due to both the polyuria and the increased chloride concentration.

The urine tests for protein during oliguria became positive during or after anoxia in the sacrifice dogs, if they were negative in the control period; or if already positive, the tests became stronger. Possibly the lack of oxygen in the blood affected the glomeruli to increase their permeability, allowing protein to escape from the blood stream; or possibly the blood flow through many glomeruli was stopped, which temporarily asphyxiated the glomerular membrane. Landis (1928) reported that oxygen lack resulting from stasis increased the permeability of the frog's mesentery capillaries and allowed protein to escape from the blood, and with the return of circulation the capillaries recovered their impermeability.

In the present work, the protein of the urine during polyuria did not change from that in the control period; the anoxia evidently was less. There was no effect of the anoxia on the urinary protein in the fistula dogs.

The tests for urinary reducing substances indicate that anoxia, under the conditions present, resulted in a temporary glycosuria during or shortly after the low oxygen. This response conforms to the epinephrine theory of anoxial oliguria. It is difficult to identify, however, why all the experiments did not result in an increased sugar excretion.

SUMMARY

Anoxia in anesthetized sacrifice dogs, induced by the respiration of gas mixtures containing percentages of oxygen below 10 per cent, for $\frac{1}{4}$ to $2\frac{1}{2}$ hours, generally resulted in oliguria, but occasionally in polyuria.

In dogs with urinary bladder-fistula, however, the anoxia resulted in polyuria, whether the animals were anesthetized or not. The presence of carbon dioxide in the inspired air altered the renal response to anoxia only under certain conditions. Several circulatory factors, particularly epinephrine secretion, were considered as possibly concerned in the changes in rate of water excretion.

The urinary concentrations of urea and chloride did not approach those of the plasma in the sacrifice or fistula dogs. The rates of urea and chloride excretion varied directly as the water excretion in both types of animals. The anoxia induced temporary proteinuria in the sacrifice animals which responded with an oliguria and a temporary glycosuria in

the majority of the experiments. In the fistula animals, anoxia was without effect on the urinary protein and sugar.

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THE EFFECTS OF EPINEPHRINE ON URINE EXCRETION IN DOGS

L. A. TOTH

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

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The oliguria which usually results after dogs have inspired gas mixtures low in oxygen (Toth, 1937) might be due to the increased secretion of epinephrine which results under these conditions. In order to determine whether epinephrine directly administered could produce urinary responses similar to those produced by the anoxia, the experiments to be reported were carried out.

The effects of epinephrine on the excretory function of the kidney have been investigated, but the data recorded are extremely conflicting; the different anesthetics, different species, and different experimental procedures confuse their interpretation. Luckhardt and Koppanyi (1927) reported that the anesthetic, and also the depth of anesthesia, influence the pressor response of the animal to subcutaneous injections of epinephrine. It was found that by using one species of animal and the same experimental procedure in a series of experiments, conclusive information could be obtained concerning the quantitative epinephrine effect on urine output, and the information obtained helps to interpret the data reported in the previous paper.

METHODS. The preparation of the dogs for the experiments was essentially the same as that already described (Toth, 1937). For injection of the epinephrine, a burette with the epinephrine solution was connected by narrow rubber tubing to a cannula in the femoral vein of the dog; judging from the rapidity of the pressor response, the dead space in the system was negligible. In one series of experiments (dog 2m) the plunger of a 1-cc. syringe was connected to a micrometer screw which was turned at a constant rate by hand. Subcutaneous and intramuscular injections (1:1000 adrenaline solution) were given all at one time.

The urine samples were analyzed for urea (plus ammonia) and chloride; protein and sugar reducing tests were also carried out on the samples. The same methods were used for these analyses and tests as were used in the previous paper.

RESULTS. *Water excretion.* Typical responses to different rates of

intravenous infusions of epinephrine in anesthetized dogs are shown in figure 1. In the experiments carried out on dog 2m, the rates of infusion varied from 1.6 to 7.7 micrograms (γ) per kilogram body weight per minute; the low rates of infusion resulted in polyuria; more rapid rates resulted in oliguria. The arterial blood pressure rose during every infusion. The total amount of fluid infused in any one animal was never more than 100 cc. Control experiments in which corresponding volumes of physiological salt solution were infused did not show any effect on the urine rate. In dog 2d (fig. 1) a rate of 6.8γ decreased markedly the rate of urine excretion although it increased the blood pressure. With a slower

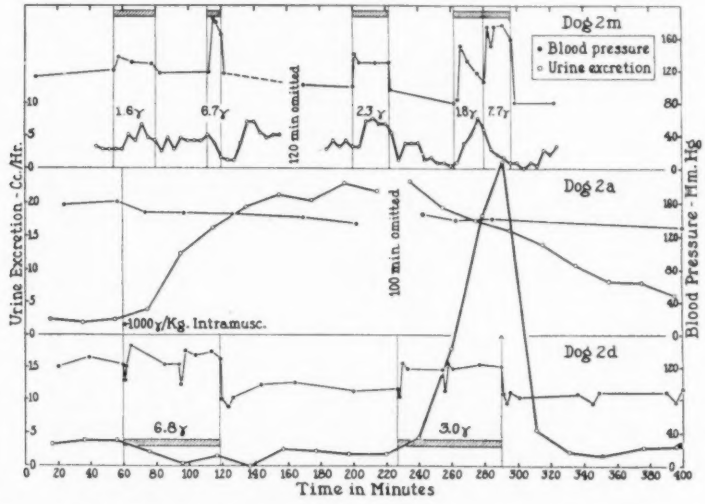

Fig. 1. Effects of epinephrine on urine excretion and blood pressure in anesthetized dogs. The numerals indicate the rates of injection, in micrograms per kilogram of body weight per minute.

rate of infusion the urinary rate increased, from approximately 2 cc. per hour to 45 cc. After stopping the infusion the urinary rate fell to the control level.

The experiment on dog 2a (fig. 1) illustrates the urinary response to epinephrine injected intramuscularly in two places. Polyuria resulted which lasted for more than six hours, even though no change in arterial blood pressure occurred. After massaging the injected areas (dog 2g-10, fig. 2, not anesthetized), the existing polyuria was interrupted temporarily. Subcutaneous injections of epinephrine (dog 2b, table 1) also resulted in polyuria, but the onset of the response was somewhat more delayed.

The data from all of the experiments carried out on dogs receiving

infusions and injections of epinephrine are recorded in table 1. In the first group of experiments the rates are from 10.3 to 5.6γ; from this rate a decided oliguria resulted; from rates of 3.0γ or less, the urinary response was polyuria. That an absolute threshold rate does not exist is shown by the experiments carried out on dog t; this animal responded with polyuria to rates of 7.7γ and 7.3γ in two experiments, but usually such rates re-

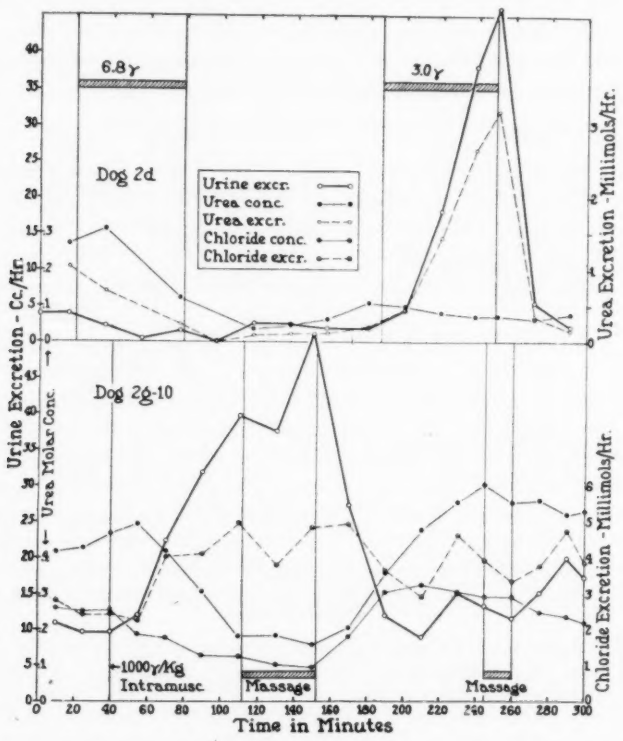

Fig. 2. Effects of epinephrine on urine excretion and solute concentration and excretion in an anesthetized and an unanesthetized dog.

sulted in oliguria. No correlation is indicated in table 1 between the rate of infusion and the onset time, or between the rate of infusion and the recovery time. The results in table 1 are similar in magnitude and duration to the urinary responses to anoxia (Toth, 1937), not only in the initial response but also in the recovery response.

In the unanesthetized dogs with bladder fistulas, large intramuscular and subcutaneous injections of epinephrine resulted in polyuria (table 1),

the intramuscular injections effecting a response in a shorter time than the subcutaneous injections. The polyurias in these experiments were of longer duration than those in the infusion experiments.

TABLE 1

Urinary responses to epinephrine in dogs

DOG NUMBER	MODE OF ADMINISTRATION	DOSAGE	DURATION	EXCRETION RATE		ONSET TIME	RECOVERY TIME	URINE RATE AFTER INFUSION, LEFT URETER
				Left ureter	Right ureter			
Anesthetized dogs								
		γ/kgm./minutes	*minutes*	*per cent**	*per cent**	*minutes*	*minutes*	*per cent**
u	Intravenous	10.3	18	0	30	10	50	100
2f	Intravenous	8.9	41	70†	45	10	10	175
2m-5	Intravenous	7.7	16	25		2	18	380
2h-1	Intravenous	7.1	25	55		10	10	195
2d-1	Intravenous	6.8	59	25	25	8	28	65
2m-2	Intravenous	6.7	8	60		2	10	135
v	Intravenous	5.6	53	35	75	28	30	95
2d-2	Intravenous	3.0	63	2,100	3,200	10	10	190
2m-3	Intravenous	2.3	22	195		6	2	105
2h-2	Intravenous	1.9	29	200		10	10	80
2m-4	Intravenous	1.8	18	880		4		
2m-1	Intravenous	1.6	25	190		2	2	120
t-1	Intravenous	7.7	37	290‡	500‡	21	4	790
t-2	Intravenous	7.3	36	260‡	360‡	27	8	280
2a	Intramuscular	1,000§		1,080		25	420	
2b	Subcutaneous	1,000§		450	370	50	280	
Unanesthetized bladder-fistula dogs								
x-12	Subcutaneous	1,000		420		130	120	
2g-9	Subcutaneous	475**		340		50	60	
2g-10	Intramuscular	475††		430		8	130	

* Percentage of normal (control) rate. Urine recovery after infusion is the maximum rate which was attained, not necessarily at "recovery time."

† Polyuria of 140 per cent preceded oliguria.

‡ Oliguria of 60 per cent during first 10 minutes of infusion.

§ Injected dose of 1000 γ/kgm. in two places.

** Massaged injected area.

†† Injected in two places and massaged areas (fig. 2).

Solute concentrations and excretions. Results of chemical analyses of the urine samples obtained from dogs responding typically to epinephrine infusions and injections are plotted in figure 2. Dog 2d was anesthetized

and received the epinephrine intravenously. Dog 2g-10 was unanesthetized and the epinephrine was injected intramuscularly. With high rates of infusion, the urea concentration of the urine decreased along with the urine excretion rate. After stopping the infusion the urea concentration gradually increased. With a rate of infusion that resulted in polyuria, the urea concentration again decreased as the polyuria set in (dog 2d). After intramuscular and subcutaneous injections of epinephrine, the urinary urea and chloride concentration in the unanesthetized dog varied inversely as the water excretion (dog 2d-10).

Glycosuria resulted from every epinephrine administration, as was to be expected, no matter what the mode of administration. After the epinephrine administration the glycosuria gradually disappeared, provided no more epinephrine was injected. Protein appeared in the urine in four of the five experiments which resulted in oliguria, this appearance

TABLE 2

Summary of chemical analyses of urine from epinephrinized dogs

NUMBER AND TYPE OF EXPERIMENT	UREA CONCENTRATION		CHLORIDE CONCENTRATION		PROTEIN	
	Decrease	No change	Decrease	No change	Increase	No change
Anesthetized:						
5 oliguria	5	0			4	1
6 polyuria	6	0			0*	4
Unanesthetized:						
3 polyuria	3	0	3	0	1	1

Figures indicate the number of experiments in which each result was found.
* Two decreased.

usually occurring after the cessation of infusion. If protein was already present, the amount increased during and after the infusion.

A summary of the chemical analyses and tests carried out on the urines from the epinephrine experiments is given in table 2. It should be mentioned that although proteinuria did not occur during polyuria resulting from epinephrine, proteinuria occurred when intramuscularly injected areas were massaged. During the massage, the polyuria was temporarily diminished, however.

Discussion. *Water excretion.* The fact that the general systemic blood pressure and the urinary response to epinephrine are not correlated in the present experiments is evident in figure 1. This lack of correlation indicates that changes in the arterial blood pressure do not explain the variation in the urine excretion.

The fourth and fifth experiments carried out on dog 2m (fig. 1) are of particular interest. Before the infusion of 1.8γ, the urinary rate was

low; this slow infusion was used in order to cause polyuria. On suddenly changing to a faster infusion, oliguria ensued.

A pressure-regulating mechanism exists locally in the kidney which must be considered in any explanation of urinary response to epinephrine. The diameter of the afferent arteriole is somewhat larger than that of the efferent arteriole, this observation being one principal factor upon which Ludwig (1861) based his filtration theory of urine formation. Provided the general systemic blood pressure does not fall, a constriction of the efferent arteriole raises the glomerular pressure. Constriction of the efferent arterioles probably increases the kidney volume; but constriction of the afferent, or of both afferent and efferent arterioles, probably decreases the kidney volume. This was suggested by Richards and Plant (1922a), who found that under the influence of minute doses of epinephrine, the blood flow through the kidneys of eviscerated cats, rabbits, and dogs was decreased at the same time that the kidney volume and urine rate increased. They also reported (1922b) an increase in kidney volume accompanied by an increase in resistance as a result of adding epinephrine to the fluid with which they were perfusing the kidney. Winton (1931) also observed that epinephrine in concentrations of 1:20,000,000 in the perfusion fluid raised the urine flow in a heart-lung-kidney preparation at the same time that the perfusion flow decreased; but higher concentrations induced a diminished excretion of urine and a greater decrease in perfusion flow, the arterial pressure being maintained constant in both cases. Doctors C. F. Schmidt and A. M. Walker (personal communication) found that intravenous injections of 10γ (total dose) in rabbits, and 20γ and 50γ in dogs, resulted in marked diminutions of renal blood flow and temporary oliguria. Within 10 minutes the blood flow recovered, sometimes to higher levels than before; occasionally polyuria accompanied the increased blood flow.

The results of the present experiments can be readily viewed in accordance with the data just cited. Infusion rates of 5.0γ/kgm./min. or higher, perhaps constrict the afferent arterioles, or both the afferent and efferent arterioles, resulting in diminution of urine flow, probably because of the decreased glomerular pressure. Infusion rates of 3.0γ constrict the efferent arterioles, raise glomerular pressure, and increase urine flow. These results are similar to those of Gunning (1917) who stated that infusions of 4.5γ/kgm./min. for 3 minutes in unanesthetized dogs resulted in oliguria; and the work of Samson and Jacobs (1932) who reported that infusions of 1γ/kgm./min. for long periods (4 to 13 days) in unanesthetized dogs resulted in polyuria. Starr (1926) also reported that epinephrine infusions of 20γ/min. for 4 minutes in anesthetized dogs resulted in oliguria while 3γ/min. for 3 minutes resulted in polyuria.

The response of glomeruli to epinephrine can be directly observed in

the frog's kidney. Richards, Barnwell, and Bradley (1927) found that dilute epinephrine in frogs' blood causes a rise in perfusion pressure and a swelling of the glomerular tufts. They interpret the result as evidence that the efferent vessels of the glomeruli were constricted. Adolph (1936) reported that epinephrine in 5γ to 25γ doses, injected subcutaneously into pithed frogs, resulted in oliguria and a brief cessation of blood flow through the glomeruli. Intravenous injections of 2γ to 4γ also caused brief oliguria, but at times such doses caused polyuria, especially when the urine rate was low.

Subcutaneous and intramuscular injections of epinephrine in massive doses did not bring about oliguria under any circumstances in the present experiments (table 1). The urinary responses seem to indicate that the absorption from these regions is at such a rate as to simulate a slow intravenous infusion. Neither injecting the epinephrine in two places, nor massaging the injected area, nor both, changed the effects to oliguria. Possibly the renal vessels became adapted to, or fatigued by, the continued influx of epinephrine and thus failed to respond to the larger concentration after massage; or the rate of destruction of epinephrine increased and kept pace with the absorption and thus maintained the epinephrine concentration of the blood at a low level. Luckhardt and Koppanyi (1927) found that repeated massage of a subcutaneously injected adrenalinized area is followed by progressively weaker responses of the pressor action, a phenomenon which they describe as fatigue of the peripheral apparatus. That subcutaneous epinephrine results in oliguria under some circumstances is indicated by Koppanyi and Dooley (1929) who reported oliguria, along with decrease in kidney volume, in anesthetized dogs after massaging the subcutaneous epinephrinized areas for 10 to 25 seconds.

Solute concentrations and excretions. Concerning the effects of epinephrine on urea excretion, Addis, Barnett and Shevky (1918) found that the rate of urea elimination from rabbits was increased after hourly subcutaneous injections of small doses of epinephrine. Watkins and Smith (1931) reported that subcutaneous injections of epinephrine into anesthetized rabbits did not influence the urea output during the first eight hours after the injection. In the present work, rapid rates of epinephrine infusion, resulting in oliguria, decreased the urea concentration and its excretion; but rates which resulted in polyuria increased the urea excretion, even though the concentration again decreased. The urinary urea concentration, however, never fell nearly as low as the blood urea concentration.

A factor which may bring about the changes in urea concentration, and subsequent decreases in urea excretion, is variation in the renal blood flow. Van Slyke, Rhoads, Hiller and Alving (1934) report that the urea clearance in unanesthetized dogs is directly proportional to the blood

flow through the kidney. In the present experiments, rapid rates of infusion of epinephrine, perhaps resulting in vasoconstriction of the afferent and efferent arterioles, may decrease the blood flow through the kidney and thus decrease the urea elimination.

The chloride concentration in the unanesthetized dogs varied inversely as the water excretion, but the chloride excretion varied directly. There seems to be no specific effect of the epinephrine on the chloride concentration or elimination. This agrees with the conclusion of Peters and Van Slyke (1932).

Temporary proteinuria usually accompanied oliguria during or shortly after the epinephrine infusion. The permeability of the glomerular membrane to protein may have increased as a result of the epinephrine itself or of asphyxiation due to constriction of the afferent and efferent renal arterioles. After stopping the infusion the arterioles may relax, but some blood protein may escape before the normal permeability is reestablished. This hypothesis was advanced by Starr (1926) and the present experiments fit it.

The effects of epinephrine on urine excretion reported in this paper are similar to the effects of anoxia on the kidney reported in the previous paper (Toth, 1937); consequently the epinephrine theory of anoxial oliguria and polyuria appears to be probable.

SUMMARY

Intravenous infusions of epinephrine into anesthetized dogs resulted in either oliguria or polyuria, depending on the rate. A rapid rate resulted in oliguria; a slow rate in polyuria. Hence both of the effects found in anoxia can also be obtained by epinephrine infusions. Intramuscular and subcutaneous injections of epinephrine resulted in polyuria. Injecting the epinephrine in two places, or massaging the injected area, had no permanent effect on the polyuria.

The urinary urea concentration decreased after rapid or slow rates of epinephrine infusions, and after injections. The urea and chloride excretion rates varied directly with the water excretion rate.

Rapid rates of epinephrine infusions which resulted in oliguria usually produced proteinuria, rates which resulted in polyuria did not affect the urinary protein content.

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DIVIDED DOSAGE OF INSULIN[1]

FRITZ BISCHOFF AND LILLIAN M. JEMTEGAARD

From the Chemical Laboratory, Santa Barbara Cottage Hospital, Santa Barbara, Calif.

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In comparing the efficacy of various insulin combinations (1) (2) (3) designed to prolong the physiologic effect, it became necessary in order to interpret the results to know with some exactness the effect of divided dosage of regular insulin.

The present report is a study of the hypoglycemia produced in rabbits by a single dose of insulin compared with the hypoglycemia produced by the same amount of insulin given in smaller doses at regular intervals.

METHODS. Ten adult rabbits were studied once weekly following a 24 hour fast. Each alternate week they received a single dose of insulin intramuscularly in an amount sufficient (1 unit per kilo or less) to produce hypoglycemia but not insulin shock. During the intervening week they received the same amount of insulin intramuscularly in divided doses. Since the divided doses produced an augmentation in effect resulting in insulin shock it was necessary to reduce the insulin dosage as the experiment progressed and repeat both the single and divided dosage procedure. Only data for animals not in insulin shock are recorded.

The experiments include the effect of 4 doses of insulin given $\frac{1}{4}$ hour, $\frac{1}{2}$ hour, or $\frac{3}{4}$ hour apart; 8 doses of insulin given $\frac{1}{2}$ hour, $\frac{3}{4}$ hour, 1 hour, or $1\frac{1}{2}$ hours apart; 16 doses given 1 hour apart; 24 doses given 1 hour apart.

RESULTS. The results are given in table 1. There is no significant difference in the hypoglycemic curves whether the insulin is given in four smaller doses during the course of $\frac{3}{4}$ or $1\frac{1}{2}$ hours, or as a single dose. Extension of the 4 dose period through $2\frac{1}{4}$ hours produces an augmentation in effect, in which comparable low blood sugar values are just two hours behind the respective control values.

Profound augmentation in effect is produced by eight doses given during the course of either $3\frac{1}{2}$ or $5\frac{1}{4}$ hours. Not only is the hypoglycemia as intense but the prolongation of effect exceeds that of the controls by four hours.

[1] This study was made possible by a grant from Mr. Wm. R. Dickinson. Some of the blood sugar determinations were performed by Mr. Russel Spicer and Mr. Willard Lee McRary.

On further division of dosage extending through 7, 11½, and 15 hours, the hypoglycemia is never as intense as that manifested by the single dose, so that one would not be justified in speaking of an augmentation of effect but rather a prolongation of effect.

The last experiment is of great interest because it shows that $\frac{1}{24}$ the hypoglycemic dose given at hourly intervals is still sufficient insulin to produce a hypoglycemia.

TABLE 1

Comparison of the effect of an intramuscular dose of insulin given in a single injection with that given in a number of smaller injections at regular intervals

TIME AND DOSE	BLOOD SUGAR AS MGM. PER 100 CC.									
	1 hr.	3 hrs.	5 hrs.	7 hrs.	8 hrs.	9 hrs.	11 hrs.	12 hrs.	13 hrs.	16 hrs.
4 injections, ¼ hr. apart	43	33	59	103						
1 injection	45	40	56	94						
4 injections, ½ hr. apart	49	37	57	107						
1 injection	42	41	60	103						
4 injections, ¾ hr. apart	65	41	47	91						
1 injection	41	46	88	113						
8 injections, ½ hr. apart	55	37	38	74		120				
1 injection	48	59	101							
8 injections, ¾ hr. apart	78	55	48	62		115				
1 injection	45	67	117							
8 injections, 1 hr. apart	92	61	54	55		67	122			
1 injection	45	75	122							
8 injections, 1½ hrs. apart	67	74	60	57		63	51		86	
1 injection	44	52	100							
16 injections, 1 hr. apart		85	84		70			63		64
1 injection	51	71	120							
24 injections, 1 hr. apart		82	78		77					
1 injection	44	52	100							

Discussion. The purpose of the present study was to establish hypoglycemic curves for divided dosage of regular insulin which would match the curves or portions of curves obtained when insoluble insulin derivatives were administered, and thus give some clue as to the rate of liberation of insulin from the insoluble combination.

For the sake of discussion and on the basis of our experience with pro-

tein hormone combinations we have classified combinations which produce delayed resorption into four groups. The insulin when injected into the tissues will, if delayed resorption takes place, do one or more of the following: 1, form or remain an insoluble compound; 2, be adsorbed or remain adsorbed by an insoluble solid; 3, be adsorbed by a naturally occurring colloid gel; 4, be entrained by precipitation of the naturally occurring tissue constituents.

It should be emphasized that as regards solubility the question of prime importance is the *period of insolubility* of the insulin combination at the point of injection, not the solubility in the solution injected. Commercial insulin is injected at pH 3. During the process of neutralization by the tissue fluids the insulin passes through its minimum solubility at the isolectric point and again becomes soluble as the pH of the tissues is approached. The fact that the insulin becomes insoluble at some point in the neutralization is not pertinent in producing delayed resorption, because the insulin does not remain insoluble for any appreciable period of time. Insulin injected at the isolectric point produces a hypoglycemic curve approximating that produced by dosage of an acid solution.

The theoretical amounts of zinc, copper, tannic acid, etc., required to form insoluble insulin combinations at pH 7.0 do not, when given with insulin, show the prolonged liberation manifested by the presence of an excess of substance. Most of the insulin in the stoichimetric combination is more rapidly liberated. The excess of precipitant must owe its effectiveness to the entrainment of the insulin compound by the precipitation of surrounding tissue constituents. (Compare 0.01 mgm. Cu with 0.1 mgm. Cu.)

	BLOOD SUGAR VALUES IN MGM. PER 100 CC. (AVERAGE FOR 5 STANDARD RABBITS)			
	1 hr.	3 hrs.	5 hrs.	7 hrs.
Control	45	50	90	110
0.01 mgm. Cu per unit	65	55	66	92
0.1 mgm. Cu per unit	107	78	59	55

The latest protamine insulin combination of Eli Lilly and Company contains 1 mgm. Zn per 500 units insulin, an amount sufficient to render the insulin insoluble at pH 7.4. The protamine might therefore be available for precipitation of tissue proteins, or as an adsorbing surface for the micell.

The hypoglycemic curves obtained in the present study indicate the proper timing of insulin resorption required to produce the augmentation effects originally reported by us (1) and now confirmed by numerous experiments on various insulin compounds. They do not, however, ex-

plain the decrease in activity observed by us for histone (3) and by Fazekas and Himwich[2] for aluminum and zinc. It is apparent that in these cases insulin liberation must greatly exceed 24 hours or some unknown factor comes into play.

SUMMARY

By injecting insulin in divided doses at regular intervals it has been possible to reproduce hypoglycemic curves resembling those obtained by injecting tissue insoluble insulin combinations.

An augmentation in effect (similar intensity of hypoglycemia with prolonged effect) is produced when the time of resorption extends over 3 to 5 hours.

A more shallow hypoglycemic curve is produced when the resorption period is extended over 7 hours.

Hourly doses of $\frac{1}{24}$ unit insulin per kilo or less still produce hypoglycemia.

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[2] *Effect of aluminum.* Data for the effect of aluminum salts on insulin resorption are given below. It will be noted that the effect is very marked. This experiment has been included because unpublished data of Bischoff and Maxwell did not agree with the report of Scott and Fischer (J. Pharmacol. Exper. Therap. **55:** 206, 1935) that aluminum was ineffective. Our results are in agreement with those recently reported by Fazekas and Himwich (J. Pharmacol. Exper. Therap. **58:** 260, 1936). The salt used in our experiment was aluminum ammonium sulfate. It is possible that Scott and Fischer based the amount of precipitant to be used on the total salt rather than the aluminum content (about 5 per cent).

	Blood sugar values in mgm. per 100 cc. (average for 8 standard rabbits)			
	1 hr.	3 hrs.	5 hrs.	7 hrs.
Control	42	47	88	110
0.1 mgm. Al per unit	86	64	77	96

DIRECT OBSERVATION OF INTRAUTERINE RESPIRATORY MOVEMENTS OF THE FETUS AND THE RÔLE OF CARBON DIOXIDE AND OXYGEN IN THEIR REGULATION[1]

FRANKLIN F. SNYDER AND MORRIS ROSENFELD

From the Department of Obstetrics and the Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University

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The present experiments represent an attempt to observe the state of activity of the fetal respiratory system by a new approach, the chief features of which are the elimination of anesthetics and the inhibition of labor. Our first experiments were undertaken because a new opportunity was offered by the hormonal inhibition of labor (Snyder, 1934) to observe fetuses in the intrauterine environment at the same stage of development as that of newborn of one, two or three days. The original problem was to determine whether pyruvic acid cyanohydrin and cyanide (Marshall and Rosenfeld, 1936) would stimulate fetal respiratory movements. The state of apnea of the fetus offered a good base-line for testing the effectiveness of a respiratory stimulant.

METHOD AND MATERIALS. In a typical experiment inhibition of labor was accomplished in a rabbit by a single intravenous injection of 100 rat units of pregnancy urine extract (Antuitrin S, Parke, Davis and Co.) administered on the 25th day of pregnancy, i.e., one week before the end of normal gestation which occurs usually at 32 days (Snyder, 1934). The spinal cord of the maternal animal was sectioned at the level of the 2nd lumbar vertebra following infiltration of the superficial tissues with 1 per cent procaine in order to permit laparatomy without the use of a general anesthetic. Incision of the abdomen in the lower midline was carried out beneath the surface of a bath of Ringer solution at 37°. The gravid uterus readily escaped from the peritoneal cavity and came under direct observation still remaining submerged. Any movements of the thorax and abdomen of the fetus could be clearly distinguished in view of the thinness and translucency of the uterine wall of the rabbit at term as well as the absence of amniotic fluid except in minute amounts.

At the conclusion of the experiment the litter was delivered and kept under observation in a warm box for 48 hours in order to determine any abnormalities.

[1] Preliminary reports: Proc. Soc. Exper. Biol. and Med. **33**: 576, 1936; This Journal **116**: 147, 1936.

Gases and volatile anesthetics were administered through a T shaped cannula introduced by tracheotomy under local anesthesia. In some experiments the complication of tracheotomy was avoided by enclosing the head of the animal in a glass chamber through which the gases could be passed. Gas mixtures were made up in 100 liter Douglas bags by admitting tank oxygen, nitrogen, and carbon dioxide through a three liter wet test meter. Check analyses indicated that the composition of a mixture, e.g., 7 per cent carbon dioxide in oxygen, lay in a range of $6\frac{3}{4}$ per cent to $7\frac{1}{4}$ per cent.

The material upon which observations were based included a total of 56 litters of rabbits which contained 220 fetuses; 14 litters of cats with 54 fetuses; and 3 litters of guinea pigs with 10 fetuses.

Rabbit fetuses were studied at various stages of pregnancy extending from 28 days to 35 days. Since the rabbits were mated in the laboratory, the duration of pregnancy was accurately known. At term, i.e., 32 days, there were 29 litters which contained 121 fetuses. At stages of prematurity there were, at 31 days, 2 litters containing 5 fetuses; at 30 days, 5 litters containing 26 fetuses; and at 28 days, an immature litter with 2 fetuses. At stages of postmaturity, there were, at 33 days, 8 litters with 33 fetuses; at 34 days, 7 litters with 17 fetuses; and at 35 days, 2 litters with 6 fetuses.

Of the 14 cats, there were 4 litters which were near term, containing 15 fetuses which weighed 90–105 grams and with crown rump length of 125–180 mm. Of the 3 guinea pigs, 1 litter was near term.

Observation. In full-term fetuses within the uterus, the respiratory system is found to be in a state of marked activity as shown by the occurrence of regular respiratory movements. Rhythmical excursions of the chest wall and diaphragm continue throughout periods of observation lasting many hours.

If now a fetus is allowed to escape into the saline bath through a small incision of the uterus while the placenta remains attached and circulation through the cord is unimpaired, the respiratory movements continue vigorously. The fetus, completely submerged in the saline bath, swims about and may crawl along the mammary region of the mother like a newborn approaching a nipple. Respiratory movements occur during periods of general body activity as well as when the fetus is at rest. With the fetus directly in view, we were able to distinguish no essential difference in the character of the fetal respiratory movements from those seen immediately after birth.

If the head of the fetus is now delivered into the air while the cord circulation remains unimpaired, the respiratory movements continue as before. With the escape of fluid from the mouth and nares, the onset of

breathing of air instead of fluid is marked by change in color of the blood of the umbilical artery from blue to bright red.

With the establishment of fetal pulmonary ventilation, the fetus now has access to two sources of oxygen supply, pulmonary as well as placental. Upon loss of the placental oxygen supply at birth, the respiratory movements, which have been traced from their origin during intrauterine life, assume significance in providing the sole mechanism of respiratory exchange.

The rate of intrauterine breathing afforded quantitative expression of the state of activity of the respiratory system, especially of the changes induced by variations in oxygen and carbon dioxide concentration of the fetal blood. Changes in depth were noted also but were more difficult to express quantitatively.

In litters which contained from one to ten fetuses there was wide difference in rate of breathing even among members of the same litter. In a single litter one or more fetuses showed respiratory movements of regular rhythm while others were completely apneic. If all of the fetuses were then detached from the uterus and removed to the air the difference which existed in the uterus was no longer evident and the entire litter after delivery breathed at a uniformly rapid rate characteristic of the newborn. Failure to demonstrate regular respiratory movements in any of the litter within the unopened uterus was encountered in about a quarter of the experiments.

In the fetuses which were delivered into the saline bath while the cord circulation remained unimpaired, the rate of respiration usually increased. In apneic fetuses respiratory movements usually set in soon after escape from the uterus into the saline bath. The rate was less rapid, however, than in the newborn.

At various stages of pregnancy it was found that a certain maximum rate was not exceeded by fetuses of a given age, although in a single litter individuals varied in respiratory rate below this level. At full term, 32 days, the intrauterine rate was about 30 per minute, in prematures at 30 days, the rate was less than half as rapid, in postmature fetuses at 34 days the rate was often twice as rapid as at term. In contrast to the antepartum respiratory rate, the newborn breathed much more rapidly, often attaining a rate of 180.

The influence upon respiration of exposure of the uterus at laparotomy and of section of the spinal cord was controlled by examination of the pregnant animal before operation. In the intact animal which was sitting quietly on a table or lying on its back, fetal movements could readily be seen by observation of the abdominal wall of the mother. At localized areas of the abdomen corresponding to the position of different fetuses

Fig. 1

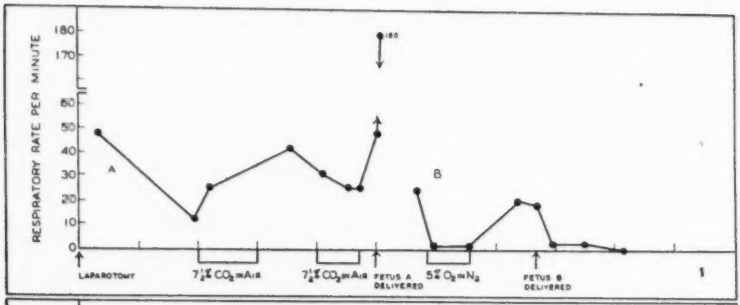

Fig. 2

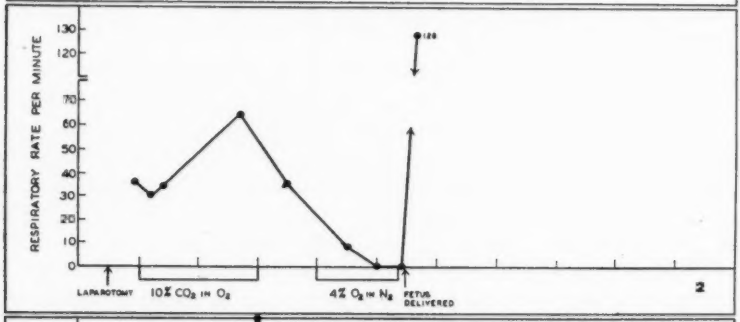

Fig. 3

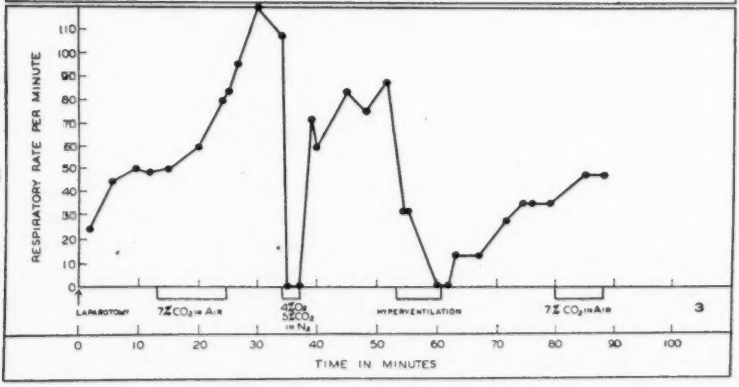

Fig. 1. Effect of carbon dioxide excess and of oxygen deficit upon intrauterine respiration of two full-term rabbit fetuses of 32 days. In fetus B, respiratory failure following experimental anoxemia.

Fig. 2. Effect of carbon dioxide excess and of anoxemia upon intrauterine respiration of a postmature rabbit fetus of 33 days.

Fig. 3. Effect of carbon dioxide excess, of oxygen deficit, and of carbon dioxide deficit upon intrauterine respiration of a postmature rabbit fetus of 34 days.

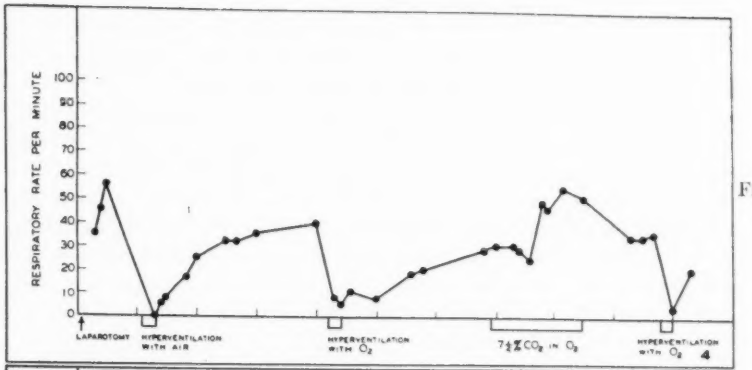

Fig. 4

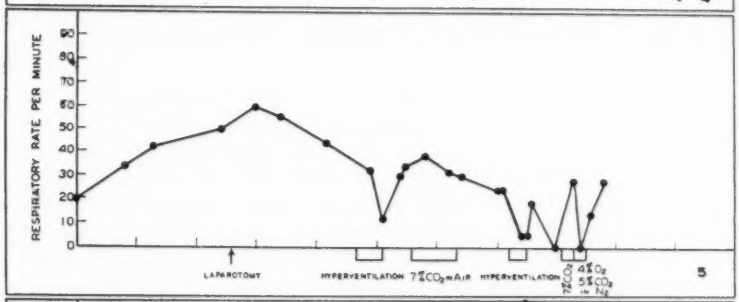

Fig. 5

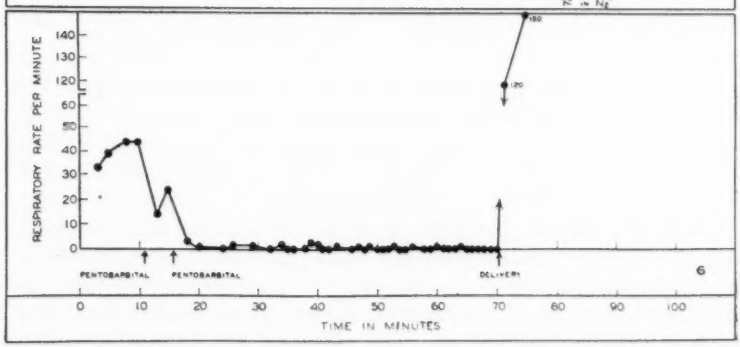

Fig. 6

Fig. 4. Effect of hyperventilation of the mother with air and with oxygen upon intrauterine respiration of a postmature rabbit fetus of 35 days.

Fig. 5. Intrauterine respiration of a full-term rabbit fetus of 32 days before and after laparotomy.

Fig. 6. Effect upon intrauterine respiration of a postmature rabbit fetus of 33 days, of narcosis with pentobarbital sodium administered intravenously to mother in 2 doses, each being 10 mgm. per kgm.

there were movements of regular rhythm at a rate which corresponded to that of fetuses observed directly through the uterine wall after laparotomy. The rate varied among individuals, and was clearly not the result of movements of maternal respiration or heart beat.

Carbon dioxide increase. If the maternal animal breathed a gas mixture containing 7½ per cent carbon dioxide in oxygen there was a striking difference in the effect on the rate of respiration in the fetus from that seen in the mother. Instead of a marked increase in rate as observed in the maternal animal, the fetus showed little or no change. In fetuses in which the respiratory rate was below the maximum characteristic of the stage of pregnancy the administration of an increased amount of carbon dioxide was followed at times by attainment of the maximum rate. An increase of carbon dioxide consistently failed to stimulate respiration to a level in excess of the maximum rate which was characteristic of the age of the fetus (figs. 1, 2, 3, 4, 5). In the majority of the fetuses there was no

TABLE 1

Effect on fetal respiratory movements of changes in blood gases

	NUMBER OF LITTERS OF RABBITS	NUMBER OF FETUSES	NUMBER OF EXPERIMENTS	RESULTS		
				Stimulation	Depression	No effect
Carbon dioxide excess	18	78	24	8	0	16
Carbon dioxide deficit	9	30	13	0	13	0
Oxygen deficit	10	53	15	0	15	0

stimulation even though the fetal rate was much lower than maximal and therefore capable of revealing any stimulant action of carbon dioxide. Fetuses were not usually aroused from apnea by 7½ per cent carbon dioxide. The results are summarized in table 1. The effect of carbon dioxide was the same whether administered in air or in oxygen.

Carbon dioxide deficit. In order to reduce the carbon dioxide tension in the maternal blood, hyperventilation was carried out by forced artificial respiration at an increased rate and depth. Excessive ventilation was continued for several minutes until apnea of the mother ensued upon cessation of artificial respiration. The effect on the fetus was striking (table 1). The fetal rate diminished sharply within a minute or two (figs. 3, 4, 5). Apnea soon followed. The interval before appearance of apnea and duration of it were parallel to the extent of hyperventilation. After cessation of artificial ventilation the fetuses remained apneic for several minutes before respiratory movements returned. Return to the rate present before hyperventilation was gradual and the fetuses regained

their initial rate more slowly than the maternal animal. The same results were noted after hyperventilation whether oxygen or air was used.

In order to control other factors than carbon dioxide deficit, which might accompany hyperventilation, forced artificial respiration was carried out with a mixture of 5 per cent carbon dioxide in oxygen. In 6 experiments in 2 rabbits with 10 fetuses, there was no evidence of depression of fetal respiration.

From the preceding observations it is evident that a certain level of carbon dioxide is essential for the maintenance of fetal respiration. An excess of carbon dioxide has little or no effect upon fetal respiration, at least in a degree comparable to that seen in the mother. A deficit of carbon dioxide results in depression or apnea of the fetus.

Anoxemia. In the present experiments the maternal animal breathed a gas mixture of low oxygen content at the same time that the rate of respiratory movements of the fetuses in the uterus was counted. There was opportunity to test by direct observation whether oxygen-want could initiate respiration in the fetus at full term. It was possible to observe the effect of anoxemia dissociated from complications such as parturition, anesthetic agents and mechanical stimulation. The results were clear cut. Anoxemia invariably depressed fetal respiration (table 1). The rate diminished under low oxygen and finally respiration ceased (figs. 1, 2, 3, 5). In striking contrast, the respiration of the maternal animal was markedly stimulated by the oxygen deprivation which coincidentally caused apnea of the fetus. The failure of anoxemia to initiate respiration was clearly illustrated in certain fetuses of the litter which were apneic at the beginning of the experiment. In no instance were respiratory movements stimulated in quiescent fetuses by oxygen-want. That these fetuses were capable of breathing was demonstrated by delivery into the air, whereupon normal respiration promptly set in.

In most experiments 5 per cent carbon dioxide was added to the gas mixture of low oxygen content in order to prevent depletion of carbon dioxide which might have resulted from increased ventilation such as occurred in the maternal animal under the stimulus of anoxemia. By this means fetal apnea which followed anoxemia was clearly differentiated from apnea which resulted from carbon dioxide deficit as described in a preceding section.

Depression of fetal respiration was evident within less than half a minute from the time of administration of a mixture containing 5 per cent oxygen and 5 per cent carbon dioxide in nitrogen. With low oxygen concentrations at various levels between 5 and 10 per cent, depression appeared but somewhat more slowly. No evidence of stimulation of rhythmical respiratory movements was noted at any level of anoxemia.

Recovery from apnea and gradual resumption of the initial rate occurred if the low oxygen mixture was removed within several minutes. The longer anoxemia was continued, the greater the incidence of fetal injury and death.

In experiments which further illustrate the depressant effect of anoxemia, rabbits at term were sacrificed by a blow on the head and the uteri were quickly extirpated. The fetuses within the excised but unopened uterus gave no evidence of stimulation of rhythmical respiration, although occasional movements of the fetal body and irregular gasps were noted. In another type of experiment following laparotomy in the saline bath and complete removal from the uterus of the placenta and unopened chorionic sac containing the fetus there was likewise no stimulation of rhythmical respiratory movements.

The effect of obstruction of the umbilical cord circulation was observed in fetuses showing regular respiratory movements in the saline bath following delivery through an incision of the uterus. Partial obstruction of the circulation was quickly followed by depression. Complete obstruction abolished respiratory movements. In apneic fetuses, sudden clamping of the cord may or may not be followed by several irregular gasps. If the fetal head is lifted from the bath into the air before the cord is clamped, gasps may result in establishment of pulmonary ventilation and coincidentally a rapid acceleration of respiratory movements.

Anesthesia. Early in the experiments it was recognized that general anesthetics profoundly influenced fetal respiratory movements. In the first attempts to observe full-term fetuses in the intact uterus we obtained in anesthetized animals confirmation of the finding of previous observers that prolonged apnea was characteristic of the fetus before birth. In rabbits in which surgical anesthesia was obtained by means of pentobarbital sodium, and the uterus was exposed in the saline bath, it was uniformly found that the full-term fetuses were in a state of apnea. However, suspicion was aroused that narcosis masked the true physiological activity of the respiratory system of the fetus. In animals in which ether was used for anesthesia, it was noted that apnea was interrupted by spontaneous respiratory movements of the fetuses at times when the depth of anesthesia was minimal.

We next tried other anesthetics of the inhalation and non-volatile groups, e.g., nitrous oxide, divinyl-ether, paraldehyde, phenobarbital, chloral hydrate in an effort to obtain an animal under standardized conditions in which fetal respiratory movements were regularly present. None of these substances proved satisfactory. In order to eliminate general anesthesia, section of the spinal cord was used.

In animals prepared in this way, a method was provided for quantitative measurement of the depressant effect of anesthetics in terms of the

effect upon the rate of fetal breathing. A finding of great significance is the extreme sensitivity of the fetus to the depressant effect of the ordinary anesthetics. For instance, intravenous injection into the mother of pentobarbital sodium (10–20 mgm. per kgm.) in amount which was insufficient to produce anesthesia or perceptibly alter respiration or reflexes of the maternal animal, nevertheless, in the fetus resulted in immediate depression or abolition of respiratory movements (fig. 6). After delivery, the depressant effect was still clearly evident in the newborn. Respiratory movements set in at birth, but at a rate less than normal. The least depressant anesthetic which we have tried is cyclopropane administered with oxygen. Deep surgical anesthesia was maintained with this anesthetic for a half hour during which time there was little or no depression of fetal respiratory movements.

Inhibition of labor. The inhibition of labor which followed the administration of a single injection of Antuitrin-S a week before term provided opportunity to observe full term and postmature fetuses in the uterine environment. Fetal rabbits corresponding in development to newborn of 1, 2 or 3 days were studied in the identical environment of the premature and term fetus, i.e., within the uterus. It was thus possible to make direct comparison of the respiratory behavior of newborn with rabbits at stages of development hitherto inaccessible to study.

In experiments with rabbits before term, i.e., at 29 or 30 days non-injected animals were used as well as those which had received 1 cc. of Antuitrin-S at 25 days. It was found by comparison that the uterus of non-injected animals when exposed in the bath was less favorable for observation of fetal respiration than that of injected ones. The quiescence and relaxation of the uterus which characterized the animals in which a new set of corpora lutea had been induced permitted uninterrupted observations of fetal respiratory movements of regular rhythm. In the non-injected animals even if labor did not intervene, the uterine motility was much increased, and was attended by wide variations in rate of fetal respiratory movements. In non-injected rabbits, despite the occurrence of active respiratory movements, the frequent fluctuations in rate prevented the attainment of a reliable basis for the comparison of factors influencing fetal respiration.

Fetal respiration in other species. Observations were extended to other species including cats and guinea pigs. In both species rhythmical respiratory movements of fetuses within the uterus were readily demonstrated. The conditions of experiment were less favorable than in the series of rabbits, since hormonal control of labor could not be employed in these species, and the stage of pregnancy was not dated by mating in the laboratory but was estimated from examination of the fetuses. Four litters of cats with 15 fetuses were near term, while the rest were at earlier

stages. About half of the animals were studied in the beginning of the experiments when ether anesthesia was employed. Apnea was present but was interrupted at times by respiratory movements, when anesthesia was light. In later experiments in which the spinal cord was sectioned instead of using ether, rhythmical respiration of the fetus within the uterus was seen in animals near term.

The occurrence of fetal respiration was also confirmed in three litters of guinea pigs. Inspection of the unopened abdomen gave evidence of fetal respiratory movements. Confirmation was obtained by exposure of the uterus at laparotomy, following section of the spinal cord. In a litter at a stage of prematurity which contained 4 fetuses averaging 35 grams in weight and 75 mm. crownrump length, i.e., about the 52nd day of pregnancy (Draper, 1920), regular respiratory movements were noted in fetuses within the uterus as well as in the extrauterine environment of the saline bath. In another litter long before the stage of viability in which there were 4 fetuses which averaged 7.5 grams in weight and 50 mm. in crownrump length, i.e., about the 38th day of pregnancy, irregular respiratory movements were noted within the uterus. When the fetuses were delivered into the bath, leaving the cord circulation unimpaired, rhythmical respiratory excursions occurred at a rate of 32 per minute. Intrauterine respiratory movements were also found in fetuses of a litter near term, the uterus being exposed under cyclopropane anesthesia.

In view of our experience in tracing the origin of certain rhythmical movements of the abdominal wall to the respiratory activity of the fetus, observations were extended to the human. In six women near term rhythmical fetal movements were clearly seen for periods of 5 to 10 minutes, occurring at a regular rate of 50 to 60 per minute. The essential characteristics of the movements corresponded to the accurate description given by Weber (1888). In two women the fetal respiratory movements were satisfactorily reproduced in a motion picture record.

Discussion.- With recognition of the activity of the fetal respiratory system, the old problem of the cause of the first breath of the newborn appears in a new light. Respiratory movements are not initiated at birth (Ahlfeld, 1905; Wislocki, 1920). The genesis of respiratory activity extends far back into embryonic life (Barcroft and Barron, 1936). Postnatal respiratory movements represent a continuation of intrauterine activity in contrast to the current view (Howell, 1936) that at birth there is an abrupt transition from a state of apnea.

The nature of fetal apnea has long been the center of curiosity in the investigation of the origin of respiratory activity (Preyer, 1885; Runge, 1905). In the present experiments we had the opportunity to trace the development of the apneic state, starting with fetuses which showed

active respiration. Three main types of fetal apnea were distinguished, namely, anoxemic, acapnic and anesthetic.

Under the standardized conditions adopted in our experiments the rate of respiration remained at a constant level for many hours. This uniformity permitted accurate evaluation of changes of rate which were induced by experimental procedures. The rôle of oxygen and carbon dioxide in the regulation of fetal respiration was revealed in a new light.

With decrease of oxygen supply the response of the fetus was in striking contrast to that of the adult animal. At a level of anoxemia which stimulated maternal respiration fetal respiration was depressed or abolished. There was no evidence that anoxemia could initiate regular respiration in apneic fetuses within the uterus, whether of full term, or of premature or postmature development.

It will be noted that the fetus bears a striking resemblance to an adult animal lacking a functional carotid body mechanism. Both in the denervated adult (Wright, 1936) and in the fetus there is absence of stimulation and accentuation of respiratory depression following anoxemia.

The response of the fetus to increase of carbon dioxide was much less pronounced than that of the adult. At a level of carbon dioxide which greatly stimulated the maternal respiration fetal respiration was not increased beyond normal limits. The slowing of fetal respiration to the point of apnea following depletion of carbon dioxide by hyperventilation of the mother established the fact that normal fetal respiration is not maintained below a certain minimal tension of carbon dioxide.

The physiological resemblance of fetal respiration to normal adult breathing is revealed by the rôle of carbon dioxide in the control of the fetal respiratory center. In comparing the chemical regulation of breathing of the fetus with that of the adult it is evident that the fetal center is less sensitive to excess carbon dioxide than that of the adult, although its response to carbon dioxide depletion shows that it is qualitatively similar to the adult.

One may note that the morphological differentiation of the fetus is marked by functional changes of respiration. The older the fetus becomes, the more rapid is its respiratory rate. In the rabbit birth is marked by abrupt acceleration to a rate which is far in excess of the intrauterine value. For instance, the newborn at full term, i.e., 32 days, breathes at a rate several times more rapid than the intrauterine fetus even though the latter is 35 days old, i.e., 3 days postmature.

In attempting to account for the variations in respiratory activity in various litters and in different fetuses of the same litter, the importance of anoxemia stands out most prominently. In our experiments the anesthetic factor was eliminated entirely, and the acapnic factor was

recognized and controlled by administration of excess carbon dioxide. This leaves the rôle of anoxemia to be considered. In this connection the relation between the state of contraction of the uterine muscle and the oxygen supply of the fetus is to be borne in mind (Anrep, 1936). Increased uterine contractility, and hence impairment of the circulation, results in depression of fetal respiratory movements. From this viewpoint it is not difficult to understand the regularity of fetal respiratory movements in the quiescent uterus under hormonal inhibition in contrast to the irregularity found in the litter of uninjected animals near term. The finding that multiparous animals are more favorable for this study than nulliparous ones, also points to significance of uterine relaxation. The occurrence of apnea in one fetus while an adjacent one in the same uterine horn shows active respiratory movements may be related to the changes in oxygen supply which accompany inequalities in the distribution of blood throughout the long tubular uterus of the rabbit. The prompt stimulation of respiratory movements in previously apneic fetuses, which follows the escape into the saline bath through an incision of the uterus, leaving the cord circulation unimpaired, may also be related to oxygen supply, in this case an increase. Finally, at birth when increase of respiratory movements is most striking, it is significant that the postnatal hypernea coincides with the acquisition of a new source of oxygen supply obtained directly by pulmonary ventilation instead of indirectly by way of placental exchange. There are other factors of course to be considered in the respiratory acceleration at birth, including the introduction of gas instead of fluid into the respiratory tract, the increase of peripheral stimuli in the new environment and the alteration of the systemic circulation of the newborn.

In previous work two chief difficulties are evident. In the first place, the direct observation under physiological conditions of a fetus in the intact uterus and of the same stage of development as the newborn has not been accomplished. Secondly, that the fetus has a special physiology has not been fully recognized. While old concepts of carbon dioxide excess and oxygen want as stimulants of respiration failed to find experimental support in the case of the fetus (Eastman, Geiling and DeLawder, 1933; Barcroft, 1936), the conditions surrounding observations of the fetus in the intact uterus were so far from physiological that the negative results were not appreciated.

Recognition of the peculiar sensitivity of the fetus to narcosis aids greatly in clarification of the findings of many earlier investigations. In the years after the introduction of anesthesia, direct observation of fetuses in the intact uterus could be made for long periods. It was regularly found that the fetuses were in a state of prolonged apnea. In the attempt to discover the cause of initiation of respiration various stimuli were

tried. Interruption of apnea by brief periods of respiratory activity had been noted, but the failure to find evidence of spontaneous fetal respiration, such as Ahlfeld had described in the human, resulted in the conclusion that respiration first begins in the newborn.

It is evident that the conclusions of previous investigations in which the cause of the first respiration was sought at birth are not easily brought into agreement with the results of our experiments (Corey, 1932; Barcroft, 1936). The clue to the disagreement lies in the conditions under which observations were carried out. In animals prepared by our method, respiration is revealed during fetal life. In contrast, in animals otherwise prepared, the fetus is usually in a state of apnea. While the interruption of fetal apnea by the first respiration appears at first to be a problem of normal physiology, it turns out in the end to be a matter of experimental technique.

CONCLUSIONS

1. The first respiration is not initiated at birth. Fetuses of the rabbit, cat, guinea pig and human show rhythmical respiratory movements within the uterus, instead of a state of prolonged apnea.

2. Experimental conditions essential for direct observation of intrauterine breathing for many hours involve the hormonal inhibition of labor and the elimination of anesthetics.

3. Regulation of fetal respiration depends upon the oxygen and carbon dioxide level of fetal blood, but differs in certain respects from that of the adult.

a. Oxygen want depresses or abolishes fetal respiratory movements.

b. Carbon dioxide deficit results in depression or apnea of the fetus showing that a certain level of carbon dioxide is essential for the maintenance of fetal respiration.

c. An excess of carbon dioxide has little or no effect.

4. The depressant action of anoxemia is evidence that carotid body mechanism is not functioning in the rabbit fetus before birth.

5. The rate of respiration of a fetus varies according to the stage of development, being several times more rapid in postmature fetuses than in premature ones.

6. The fetal respiratory system is peculiarly sensitive to narcosis. Respiratory movements may be abolished in the fetus at a level of analgesia which does not impair maternal respiration.

7. Prolongation of pregnancy by the inhibition of labor made possible the comparison of the fetus in the intrauterine environment with the newborn of 1, 2 or 3 days, both being at an identical stage of development. The quiescence and relaxation of the uterus under hormonal influence favored continuation of respiratory activity without interruption by circulatory disturbance.

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A COMPARISON OF THE METABOLIC EFFECTS OF SUBCUTANEOUS AND INTRAVENOUS EPINEPHRINE INJECTIONS IN NORMAL AND HYPOPHYSECTOMIZED RATS

JANE A. RUSSELL AND GERTY T. CORI

From the Department of Pharmacology, Washington University School of Medicine, St. Louis, and the Institute of Experimental Biology, University of California

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That epinephrine produces only slight or no glycosuria in well-fed as well as in fasting hypophysectomized dogs was first stated by Aschner in 1912 (1). Since then a number of investigators have found that subcutaneous administration of epinephrine does not cause so great a rise in blood sugar in hypophysectomized dogs, rabbits or toads as it does in normal animals (2, 5, 6, 10, 13, 14, 15) supposedly because the liver is less responsive to the glycogenolytic action of epinephrine in the absence of the hypophysis (10, 14, 20). The changes in blood lactate (5, 15) and inorganic phosphate (5) caused by subcutaneous epinephrine administration were found to be diminished after hypophysectomy and it has been suggested that this alteration in the action of epinephrine may be due to a decreased glycogenolysis in muscle. Bachman and Toby (2), on the basis of some muscle glycogen determinations in rabbits, also assumed that the responsiveness of muscle glycogen to epinephrine may be diminished after hypophysectomy.

In the following instances epinephrine was given intravenously. Braier (4) found that with this mode of application epinephrine produced identical effects on the blood sugar levels of normal and hypophysectomized dogs when they were fasted 18 hours. Marks (15) noted that the changes in muscle glycogen due to intravenous injection of epinephrine were about the same in a small series of normal and hypophysectomized cats. However, Fluch, Greiner and Loewi (12) found diminished glycogenolysis in perfused livers of frogs from which the pars posterior had been removed. The authors state that these frogs developed no atrophy of the adrenal cortex. Since hypophysectomized mammals (as well as hypophysectomized amphibia (18)) develop atrophy of the adrenal cortex these experiments apparently deal with animals in which the internal secretion of that part of the pituitary which is equivalent to the anterior lobe in mammals had not been abolished.

The claim made by several workers, that removal of the hypophysis

diminishes or even abolishes the glycogenolytic action of epinephrine is founded on experiments in which epinephrine was administered subcutaneously. It seemed possible that the rate of absorption of epinephrine from the subcutaneous tissues of hypophysectomized animals might be diminished. This was tested by making a comparison of the effects of subcutaneous and intravenous epinephrine injections.

EXPERIMENTAL. All the animals used were young male rats weighing from 150 to 200 grams, and, when hypophysectomized, used from 10 days to 4 months after the operation. The glycogen determinations were made by the cold KOH method (7). The non-fermentable reducing substances were removed from the urine samples (19). Sugar was determined with the Shaffer-Somogyi copper reagent containing 1 or 5 grams KI. For hexosemonophosphate the method of Cori and Cori was used.

The intravenous infusions were made either with the injection pump described by Cori, Fisher and Cori (8) or with the Burn-Dale burette. Catheter tubing was used to connect the syringe or burette with the injection needle. In all cases in which epinephrine was infused, glutathione was added (2 mgm. per 10 cc. of infusion fluid) to prevent inactivation of the epinephrine in vitro. Two and one-fifth to 3 cc. were injected per hour in all cases. Glucose was administered in a 12 to 15 per cent solution, the exact amount infused being determined by analysis of samples delivered by the infusion device.

The usual procedure in the infusion experiments was as follows: The rat was anesthetized with amytal or nembutal injected intraperitoneally and was kept anesthetized, but not too deeply, during the rest of the experiment by additional small doses of the anesthetic as required. In some experiments blood (0.2 cc.) was drawn from the jugular vein; in others one gastrocnemius was removed for analysis. Hemorrhage was easily controlled by applying hemostats. The femoral vein was exposed (on the side on which the muscle had been excised), the needle (size no. 25) from the infusion apparatus was inserted and arranged to stay in place without ligatures, and the infusion was started. Rectal temperatures were taken at intervals during the experiments and maintained between 37 and 38°C. by supplying heat with an electric bulb. If the respiration was markedly slowed during the infusion, the experiment was discarded. At the end, additional anesthetic was given if necessary, the other gastrocnemius removed for analysis, a blood sample taken from the vena cava, and the liver removed for glycogen determination. If glucose had been infused, the bladder was emptied as completely as possible by gentle pressure from inside the abdominal cavity, and the urine excreted throughout the experiment was analyzed for glucose.

In experiments in which epinephrine was given subcutaneously, all details were identical except for the actual administration of the epineph-

TABLE 1

Effect of epinephrine on blood sugar and muscle glycogen of normal and hypophysectomized rats

The experiment lasted 1 hour. The animals were not fasted, except as noted.

RAT	BLOOD SUGAR IN MGM. PER CENT			MUSCLE GLYCOGEN IN MGM. PER CENT		
	Before	After	Difference	Before	After	Difference
A. No epinephrine						
Normal	114	103	−11	768	777	+9
Hypophysectomized	140	128	−12	553	521	−32
Hypophysectomized*	139	123	−16	580	591	+11
Hypophysectomized*	137	136	−1	703	683	−20
Incompletely hypophysectomized		113		604	592	−12
Incompletely hypophysectomized		72		545	556	+11
			−10	626	620	−6
B. 0.02 mgm. epinephrine per 100 grams subcutaneously						
Normal	108	340	+232	743	590	−153
Normal	115	232	+117	498	360	−138
Normal		160		594	373	−221
Normal	96	135	+39	482	326	−156
Normal	113	167	+54	548	295	−253
Incompletely hypophysectomized†	85	157	+72	577	406	−171
			+103	574	392	−182
Hypophysectomized	110	134	+24	654	637	−17
Hypophysectomized	125	188	+63	586	490	−96
Hypophysectomized	126	156	+30	603	560	−43
Hypophysectomized	111	134	+23	712	636	−76
Hypophysectomized	116	152	+36	630	584	−46
			+38	637	581	−56
C. 0.0025 mgm. epinephrine per 100 grams per hour intravenously						
Normal	110	216	+106	725	620	−105
Normal	148	191	+43	595	447	−148
Normal	142	178	+36	484	330	−154
Normal	108	205	+97	536	396	−140
Normal	124	184	+60	495	395	−100
			+68	567	437	−130
Hypophysectomized	128			674	515	−159
Hypophysectomized	158	198	+40	668	516	−152
Hypophysectomized	117	250	+133	641	465	−176
Hypophysectomized	115	162	+47	550	386	−165
Hypophysectomized	138	231	+93	563	426	−137
			+78	619	461	−158

* Saline infusion.

† Fasted 18 hours.

TABLE 2

Effect of epinephrine on muscle hexosemonophosphate of normal and hypophysectomized rats

The animals were not fasted

SUBCUTANEOUS EPINEPHRINE, MGM. PER 100 GRAMS	BEFORE INJECTION HEXOSEMONOPHOSPHATE, MGM. PER CENT			1 HOUR AFTER INJECTION HEXOSEMONOPHOSPHATE, MGM. PER CENT			BLOOD SUGAR, MGM. PER CENT
	Hexose	P found	P calc.	Hexose	P found	P calc.	
			Normal				
0.02*	38		7	84		15	222
0.02	34	4	6	62	7	11	227
0.02	42	9	7	78	14	13	179
0.04	46	7	8	102	18	17	245
	40		7	83		14	218
			Hypophysectomized				
0.02	31	4	5	42	7	7	98
0.02	53	7	9	68	10	12	111
0.02	33	4	6	50	7	9	71
	40	5	7	53	8	9	93
			Normal				
INTRAVENOUS EPINEPHRINE, MGM. PER 100 GRAMS PER HOUR							
0.014	57	9	10	101	15	17	212
0.007	42	6	7	116	22	20	220
0.006	49	8	9	88		15	301
0.006	41	7	7	86	14	15	225
0.004	43	7	7	124	19	21	169
0.003	52	7	9	83	15	14	184
0.0066	47	7	8	100		17	218
			Hypophysectomized				
0.008	45	5	8	119	17	21	166
0.007	58	7	10	124	20	21	250
0.006	65	8	11	134	18	23	227
0.003	51	7	9	122	20	21	228
0.006	55	8	9	125	19	21	218

* Incompletely hypophysectomized.

rine; it was injected in a volume of approximately 0.2 cc. immediately after the first muscle sample was removed or the glucose infusion was started. In control experiments, either nothing was administered, or as indicated in table 1, section A, a blank infusion was performed.

Once the liver glycogen is depleted in hypophysectomized rats (which is usually the case during the early part of the fast), rapid changes may occur in the muscle glycogen levels and so prevent an accurate comparison of the effects of the epinephrine in the control and hypophysectomized animals. For this reason the animals used in the experiments in table 1 were not fasted prior to the experiment. Since usually a small drop in blood sugar occurred in control experiments (see table 1, section A), it was not considered that absorption from the intestine influenced the results to an appreciable extent. In the experiments on glucose tolerance (table 3), the aim was to have approximately equal glycogen levels in the two groups of animals. Taking into account the difference in the rate of disappearance of glycogen (11), the hypophysectomized rats were fasted for 10 and the normal animals for 20 hours prior to the experiment.

Effects of epinephrine on muscle glycogen and hexosemonophosphate. As shown in section B, table 1, the changes in muscle glycogen due to epinephrine were much less in the hypophysectomized rats than in the normal when it was given subcutaneously; but when it was given intravenously (section C, table 1), the effects were, if anything, somewhat more marked in the hypophysectomized series. It can be concluded therefore that in these experiments the absence of the pituitary did not diminish the response of muscle glycogen to epinephrine.

While glycogen disappears from muscle during epinephrine action, hexosemonophosphate accumulates (9). Table 2 shows that this accumulation occurred in the absence of the pituitary, provided the epinephrine was administered intravenously. After subcutaneous application of epinephrine hypophysectomized rats showed, concomitant with small changes of muscle glycogen, only a slight rise in hexosemonophosphate.

Effect of epinephrine on blood sugar. The averages in tables 1 and 2 show that the rise in blood sugar is less in the hypophysectomized rats when the epinephrine is given subcutaneously, while it is practically the same as in the normal rats when the epinephrine is infused intravenously.

One of the factors which may influence the hyperglycemic action of epinephrine is the liver glycogen level; for while that level can vary within a wide range without exerting much effect, when it is very low the hyperglycemic effect of epinephrine is necessarily much reduced. In hypophysectomized rats fasted for short periods such as 8 hours, the liver glycogen has almost disappeared (17) and such animals are therefore not suited for a study of the effect of epinephrine on blood sugar. Since very low liver glycogen levels are also found in other species of hypophysectomized animals, when they are fasted, this factor must be known accurately before the results of epinephrine administration can be interpreted. In the present experiments the liver glycogen level probably did not appreciably influence the results, for though it varied considerably

because the animals were not fasted, it was almost always relatively high. The average values for liver glycogen at the end of the experiments in table 1 were for section B, normal 1.24, hypophysectomized 1.24; for section C normal 1.70 per cent, hypophysectomized 0.47 per cent.

Effect of epinephrine on glucose tolerance.[1] In table 3, 176 mgm. of glucose per 100 grams per hour were given to the hypophysectomized and 226 to the normal animals, the infusions lasting 2 hours. The resulting blood sugar curve showed a greater rise in the former than in the latter animals. The reduction of approximately 30 per cent in carbohydrate uptake indi-

TABLE 3

Effect of epinephrine on the intravenous glucose tolerance of normal and hypophysectomized rats

The infusions lasted 2 hours in each case

NUMBER	NUMBER OF ANIMALS	GLUCOSE INFUSED, MGM. PER 100 GRAMS PER HOUR	GLUCOSE EXCRETED IN 2½ HOURS, MGM. PER 100 GRAMS	BLOOD SUGAR, MGM. PER CENT				INCREASE DUE TO EPINEPHRINE*
				0 hour	1 hour	2 hours	2½ hours	
No epinephrine:								
Normal	5	226	8	97	265	347	169	
Hypophysectomized	5	176	0	63	321	384	227	
Subcutaneous epinephrine, 0.02 mgm. per 100 grams:								
Normal	4	222	108	77	376	542	432	*195*
Hypophysectomized	5	173	4	75	349	493	346	*109*
Intravenous epinephrine, 0.001 mgm. per 100 grams per hour:								
Normal	4	225	36	83	369	500	292	*153*
Hypophysectomized	4	174	12	84	356	568	422	*164*
Intravenous epinephrine, 0.005 mgm. per 100 grams per hour:								
Normal	3	223	120	88	440	582	416	*235*
Hypophysectomized	3	172	34	81	468	734	540	*350*

* Difference in blood sugar values for 2 hour period, f.i., 542 − 347 = 195.

cated by this comparison is probably due, in part at least, to the depression (usually 30 to 40 per cent) in the metabolic rate of the hypophysectomized animals (11).

The increase in the blood sugar levels after subcutaneous administration of epinephrine was greater in the normal than in the hypophysectomized rats (see last column, table 3). However, when the epinephrine was given intravenously with the glucose, the resulting blood sugar levels were higher in the operated than in the normal rats.

The excretion of sugar in the urine did not prove to be a good index

[1] These experiments were performed in collaboration with Dr. R. E. Fisher.

of the magnitude of epinephrine action, because the hypophysectomized rats, owing to some disturbance in kidney function, excreted only $\frac{1}{2}$ to $\frac{1}{25}$ as much sugar as did the normal rats. Consequently, the blood sugar curve would have been lower in the hypophysectomized rats given epinephrine subcutaneously, had the excretion of sugar in the urine not lagged so far behind, and conversely the blood sugar curve with intravenous injection of epinephrine would not have reached such high values had the kidney function not been deficient. The nature of the disturbance in sugar excretion in hypophysectomized animals remains obscure at the present time. The fact remains, however, that subcutaneously administered epinephrine produced less effect on glucose tolerance in the hypophysectomized than in the normal animal, while there was no marked difference with intravenous administration.

The averages for liver glycogen at the end of the glucose infusion (without injection of epinephrine) were 1.80 for the normal and 0.71 per cent for the hypophysectomized, which indicates a deficiency in the formation of liver glycogen in the latter animals, an observation which has been recorded previously by Phillips and Robb (16) and by Bennett (3). After subcutaneous injection of epinephrine plus glucose, the average liver glycogen was 2.13 grams in the normal and 1.02 per cent in the hypophysectomized animals, and similar values were found in the two series with intravenous injection of epinephrine.

Discussion. In the experiments presented above, the effects of an intravenous epinephrine injection on muscle glycogen and hexosemonophosphate, blood sugar and glucose utilization were practically the same in normal and hypophysectomized rats. The differences in the reaction of the two types of animals to subcutaneously injected epinephrine are therefore attributed to a decreased rate of absorption of the epinephrine from the subcutaneous tissues of the hypophysectomized rat. It seems probable that the low blood pressure and poor circulation through the skin and possibly also the low metabolic rate of these animals are responsible for this phenomenon.

These results suggest that in other species as well, the rate of subcutaneous absorption may be important in determining the effects of epinephrine in the absence of the hypophysis. An interpretation of such effects can hardly be made unless account is taken of the absorption factor.

SUMMARY

1. The effect of epinephrine on muscle glycogen, hexosemonophosphate, blood sugar and intravenous glucose tolerance of anesthetized normal and hypophysectomized rats was determined. When the epinephrine was given subcutaneously, all of these effects were less in the hypophysec-

tomized than in the normal rats; but when the epinephrine was given in a slow intravenous infusion, the effects in the hypophysectomized animals were equal to those in the normal ones. The differences in the reaction of the normal and hypophysectomized rats to the subcutaneous injection of epinephrine are therefore attributed to a diminished rate of absorption of the epinephrine from the subcutaneous tissues in the absence of the hypophysis.

2. The hypophysectomized rats showed a lower tolerance for intravenously administered glucose than did the normal ones, the reduction being of about the same order of magnitude as the decrease in metabolic rate.

3. The hypophysectomized rats exhibited an abnormally high renal threshold for glucose, and whether epinephrine was given or not, excreted less glucose than did the normal rats.

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NUSSBAUM'S EXPERIMENT ON RENAL SECRETION[1]

RUDOLF T. KEMPTON

From the Laboratory of Pharmacology, University of Pennsylvania, Philadelphia

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Nussbaum's classical experiments (1) purported to show that the tubules of a frog's kidney, in which glomerular circulation had been abolished by ligation of the renal arteries, possessed the capacity of transferring dissolved substances (dyes) from the blood into the lumen of the tubule and of responding to the presence of urea in the blood by excretion of significant amounts of urine into the bladder. They provided the most convincing support then available of the hypothesis that the epithelium of the renal tubule secretes urine. Subsequent work has established the validity of the observation that substances may gain access to the urine through the tubule wall: decisive confirmation or refutation of the second aspect of Nussbaum's work, viz., the elimination of urine by the frog's kidney in the absence of glomerular function, has not, in our opinion, been supplied. It is this item of the subject with which this paper deals.

Adami (2) repeated Nussbaum's experiments using a dorsal rather than a ventral approach to the kidney and concluded that glomerular circulation was stopped only for a time by arterial ligation, a collateral supply to the glomeruli then being established. Beddard (3) showed that Adami's method did not permit the destruction of all arteries reaching the kidneys: Bainbridge and Beddard (4) duplicated Nussbaum's results; they found however that it was necessary to keep their animals in an oxygen atmosphere to prevent degeneration of the tubular epithelium. As proof that glomerular circulation had been completely stopped they stressed the observations that the glomeruli became plugged with blood clots which were not washed out by subsequent saline perfusion, and that dye solutions injected into the aorta failed to reach any glomeruli. Bieter and Hirschfelder (5) ligated the arteries to parts of the kidney, saw that glomerular circulation stopped, later to be reëstablished through (undescribed) collaterals; they expressed the thought that this must have occurred in Nussbaum's experiments. They later (6) found that after ligation of all visible renal arteries to one kidney a few arterioles in the lower half still showed pulsating flow: and after ligation of the renal portal vein a pulsating stream near the point of ligature was seen in it. Tamura et al. (7) ligated the renal arteries

[1] The expenses of this work were defrayed in large part by a grant from The Commonwealth Fund.

of the Japanese frog, Rana nigro-maculata, and found that no urine was produced. However their animals were kept under urethane narcosis throughout the experiment and were observed no longer than five hours. Bainbridge and Beddard had found that no urine was produced the first day after the operation, and urethane is known to depress the circulation. In the Japanese toad Tamura and his co-workers (8) ligated all but one of the arteries of one kidney, but the experiments were not designed to show whether or not urine was formed by the tubules without functioning glomeruli. Bensley and Steen (9) ligated some of the frog's renal arteries and reported that the circulation in the corresponding glomeruli was effectually stopped. They confirmed Nussbaum's observation of secretion of injected dye by the epithelium of the second section of the tubule but their description does not indicate that fluid was eliminated from the kidney after abolition of its glomerular circulation. Gerard and Cordier (10) noted the reëstablishment of glomerular circulation following arterial ligation in the toad, but this did not occur in less than ten days after the operation. They found no injury of the tubular epithelium. Recently Adolph (11) has shown that under conditions of asphyxia glomerular circulation is completely arrested in the frog and complete anuria occurs. Although he refers to this as the "Nussbaum experiment performed without surgical intervention," lack of oxygen might be expected to suppress completely all secretory activity of the tubular epithelium.

Some evidence has also accumulated which indicates that blood can reach the glomeruli via the renal portal system. Richards and Walker (12) showed that during double perfusion the renal portal perfusion fluid reached many glomeruli although its pressure was only a fraction of the aortic: and Bieter and Hirschfelder (6) saw pulsating blood flow in the renal portal vein after it had been ligated. They did not determine its source.

It will be noted that the only exact repetition of Nussbaum's experiment resulted in complete confirmation (Bainbridge and Beddard). On the other hand, fragmentary evidence obtained by different methods indicates that urine is not excreted when there is no glomerular function. In 1926 Cushny (13) wrote "The secretion under urea is generally regarded as proving definitely that urea is normally secreted by the tubules, and as offering almost conclusive evidence in favor of Heidenhain's view of renal secretion."

In the present work scrupulous effort has been made to reproduce the conditions of Nussbaum's experiment in American frogs in the hope that his results, if duplicated, could be subjected to further critical study. The results have convinced us that the elimination of urine from the kidney in Nussbaum's experiments was the result of glomerular function, not of tubular activity.

MATERIAL AND METHODS. Male specimens of the American bullfrog, Rana catesbiana, were used for the most part. The operative method of Nussbaum was carefully followed in all details, except that ether anesthesia and the electrocautery were used in the operation. After the first experiment, it was found necessary to use a precaution suggested by

Bainbridge and Beddard, i.e., destruction of small arteries reaching the anterior pole of the kidney from the coeliaco-mesenteric artery and from the angle between this and the aorta. In the final experiments additional ligatures were applied, as is described below. In some experiments the animals were kept in an oxygen atmosphere following the operation.

The details of the operation were as follows: After the animal was etherized, the bladder was emptied by catheter. A longitudinal skin incision was made in the mid-ventral line of the abdomen, the abdominal vein doubly ligated and divided, and the incision continued toward the groin on each side. The bladder was examined to make certain it had been emptied, and a ligature was placed around the rectum as far posterior as possible. The organs were displaced, and the peritoneum torn between the lateral border of the kidney and the body wall. The dorso-lumbar branch of the renal portal vein was then doubly ligated and divided, and the kidney lifted to expose the renal arteries, which were then ligated. The procedure was repeated on the other side so that no arteries should be missed. A cannula for subsequent intravenous injections was inserted into the anterior abdominal vein and the wound closed with the cannula projecting from it. After again catheterizing the bladder a purse-string ligature, previously passed around the cloaca, was tied securely. The animal then received an intravenous injection of 4 cc. of a 10 per cent solution of urea in 0.65 per cent NaCl and was placed in water in an air or an oxygen atmosphere. Recovery from ether occurred in a few minutes. At intervals the frog was lightly etherized, the cloacal ligature loosened, a catheter introduced into the bladder to collect any urine which had formed, and the ligature again tightened.

After one or two days an examination of the living kidney was made. The animal was narcotized with urethane (2 grams per kilo), opened, and prepared in the usual manner for microscopic examination of the kidneys by reflected light. After the desired study had been made, the kidney was washed free of blood by perfusing Clark's solution through the aorta at a pressure of 56 cm., according to the method of Bainbridge and Beddard. This was followed by an injection at the same pressure of a saturated solution of Berlin blue (Grübler) in Clark's solution for 10 to 30 minutes. The entire animal was then placed in absolute alcohol, and this was frequently changed. When it was desired to section the kidneys they were removed after a half-hour and transferred to fresh absolute alcohol.

RESULTS. 1. *Nussbaum's results confirmed.* In experiments such as are summarized in the following tabulation it was found that urine was excreted in response to intravenous injections of urea by kidneys which had been subjected to complete arterial ligation according to Nussbaum and Bainbridge and Beddard.

FROG NUMBER	WEIGHT	10 PER CENT UREA INJECTED	URINE FROM BLADDER	HOURS
	grams	*cc.*	*cc.*	
1	293	None	None	48
2	174	4	4.6	21
3	222	4	18.9	22
4	128	4	0.11	21
8 (control)	169	4	33.4	24

In experiments 2 and 4 hemorrhage occurred during the operation: there was no hemorrhage in experiment 3. Experiment 8 was a control in which the entire operation was performed without however tying the ligatures which had been placed about the renal arteries.

2. *Glomerular circulation after ligation of all renal arteries.* In the above experiments and others like them the kidneys were exposed for examination during life on the day following the operation. Despite the fact that all the renal arteries were found ligated, circulation was visible in glomeruli in the posterior pole of the kidneys (figs. 1 and 2). In experiment 1, in which no urea was injected, it was extremely sluggish; in experiments 2, 3 and 4 it was very vigorous. The only animal in which

Figures. Diagrams of ventral views of the kidneys of frogs whose renal arteries were ligated. The arteries are indicated by stippling, the other vessels are veins. Arrows indicate the direction of flow of the blood.

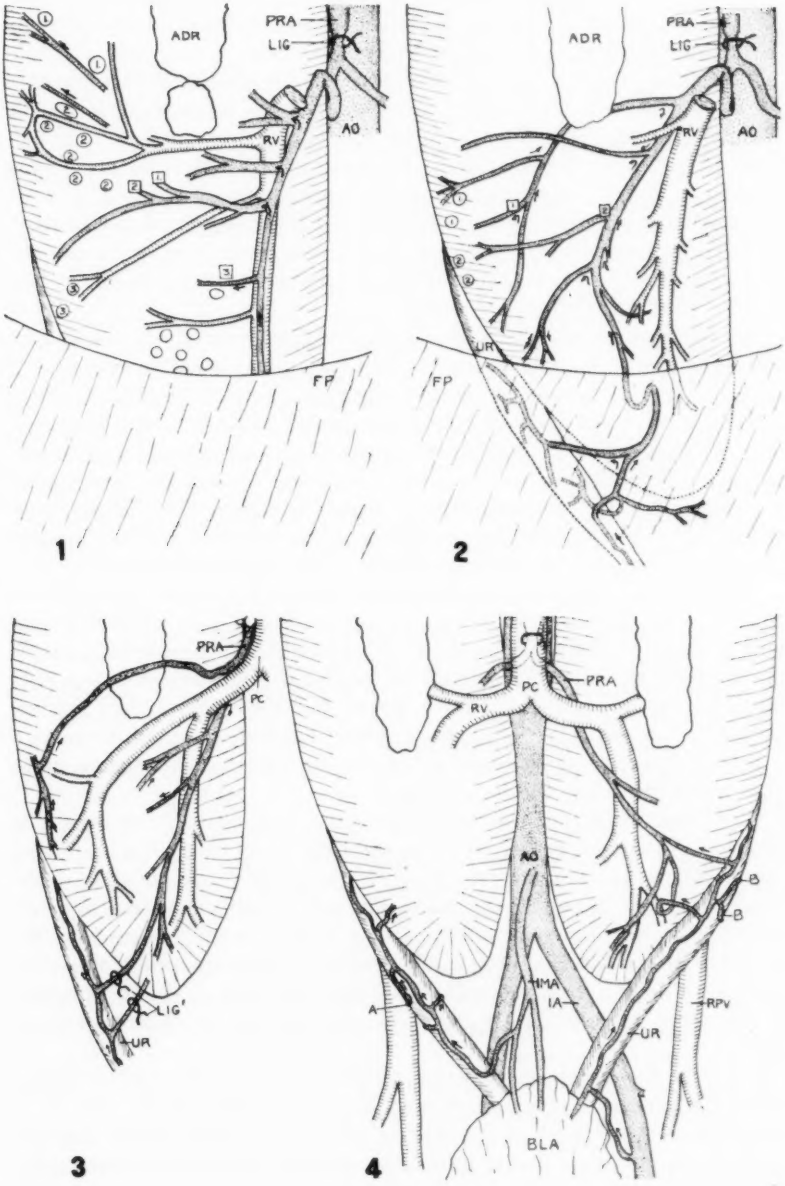
ADR, adrenal gland; *AO*, aorta; *BLA*, bladder; *FP*, fold of peritoneum covering the posterior part of the kidney; *IA*, iliac artery; *IMA*, inferior mesenteric artery; *LIG*, effective ligature around posterior renal artery; *LIG'*, additional effective ligature to suppress collateral circulation; *PC*, posterior vena cava; *PRA*, most posterior renal artery; *RPV*, renal portal vein; *RV*, renal vein; *UR*, ureter.

Fig. 1. Right kidney of frog 3. The fold of peritoneum is indicated unnaturally far posterior in this and in figure 2. In both figures the circles indicate glomeruli through which circulation was maintained. The squares indicate points at which pressure on the artery stopped circulation in the correspondingly numbered glomeruli.

Fig. 2. Right kidney of frog 4. The artery on the fold of peritoneum is indicated by solid lines, but where it leaves the surface to connect with the kidney and with the ureter it is indicated with broken lines.

Fig. 3. Right kidney of frog 12, showing the position of the additional ligatures used to suppress the major part of the collateral circulation. This circulation was completely suppressed at the posterior end, but more anteriorly blood entered the kidney as indicated by the arrows.

Fig. 4. Both kidneys of frog 11. *A* indicates the cross connection between the vessel on the wall of the ureter and the renal portal vein. When the inferior mesenteric artery was clamped blood flowed into the kidney from the renal portal vein through this connection. The branches *B* are similar connections on the other side. The right kidney was supplied by the inferior mesenteric, while the left was supplied by a branch from the left iliac artery.


ADR
PRA
LIG
RV
AO
FP
1
ADR
PRA
LIG
RV
AO
UR
FP
2
PRA
PC
LIG
UR
3
PC
PRA
RV
AO
B
B
HMA
IA
A
RPV
UR
BLA
4

Figs. 1–4

no glomerular circulation could be detected was one in which hemorrhage during and following the operation had been excessive and in which no urine was excreted into the bladder.

The most posterior renal artery to each kidney is one of two branches of a single vessel arising from the aorta. Each of these after passing ventrally between the kidneys, turns onto the ventral surface of the kidney which its branches supply. Its main trunk, extending posteriorly on the ventral surface, leaves the surface near the posterior end and passes onto a fold of peritoneum overlying the posterior part of the kidney. Continuing posteriorly it gives branches to the ureter which, in the male, is here enlarged to form the seminal vesicle and to the peritoneum and mesentery which support the ureter.

In the kidneys of frogs with ligated renal arteries blood was seen to be flowing in this main trunk of the most posterior renal artery in a direction the reverse of the normal (figs. 1 and 2). The artery was so enlarged that when first seen it was considered to be a vein, and this error was not revealed until the perfusion of the kidney and the subsequent dissection. The blood passed from this vessel out through its branches in a normal direction into the afferent vessels and glomeruli. Blood from the ureteral vessels therefore reached the glomeruli through the posterior renal artery and its branches peripheral to the point of its ligation.

When the kidneys were observed at the time of ligation of the posterior arteries, this reversal of flow was seen to occur at once; the flow was sluggish, and took place even if the other renal arteries were not ligated. In these freshly prepared animals the source of blood was a coarse network in the ureteral wall, which before ligation was supplied by the posterior renal artery. The day after the ligation, however, there was a large channel carrying blood along the wall of the ureter from more posterior regions into the renal artery.

The blood supply to the ureter was then examined. It was usually found that the ureter received blood from the aorta through a branch of the inferior mesenteric artery. That this is not the only possible source was shown by one animal (fig. 4) in which the right ureter was supplied as usual by the inferior mesenteric artery, while the left was supplied by a branch of the left iliac artery. In another animal the ureter was supplied by a branch from the wall of the posterior part of the rectum and in this there was flow in a reversed direction through the posterior renal artery.

Evidence was obtained from two animals that renal portal blood may reach the posterior renal artery and glomeruli. In frog 11 (fig. 4) a rather large side branch in the wall of the ureter carried blood dorsally to the adjacent renal portal vein. Occasionally there was spontaneous temporary reversal of flow in this short connection, so that renal portal

blood entered the posterior renal artery along with the arterial. When the inferior mesenteric artery was clamped, blood flowed from the renal portal vein into the posterior renal artery. In another frog (no. 15) there was reversal of flow in one of the anterior arterioles. When the renal portal vein was clamped at the posterior end of the kidney the flow in the anterior arteriole was brought to a standstill, indicating that it had been supplied by renal portal blood.

The basis of the collateral circulation supplying the glomeruli is therefore the double arterial supply of the vascular bed in the wall of the ureter. When one source of arterial supply to this bed is stopped by ligation, the other source of blood furnishes its entire supply. Since between the ligature of the posterior renal artery and this vascular bed there are open branches passing into the kidney, the pressure relationships are such that the blood flows from the ureter into the posterior renal artery and out through its renal branches. In the course of several hours this pathway becomes modified so that relatively large channels are formed along the ureteral wall, in which the originally sluggish flow becomes rapid and pulsating.

3. *Effect of urea upon the collateral circulation.* Since in these experiments, as in those of Nussbaum, urine was formed only after the injection of urea, it is important to examine the effect of urea upon the collateral circulation. In one animal subjected to the usual operation under urethane narcosis, the kidney was examined before any injections were made. The venous flow in the kidney was brisk and there was the usual sluggish reversed flow in the posterior renal artery. No glomerular circulation could be detected in the left kidney, and only three open glomeruli could be found in the right. The animal was then covered with moist cotton and allowed to remain undisturbed. An hour later no glomerular circulation could be seen. Four cubic centimeters of Clark's solution were then injected intravenously, this being the volume of the urea solution usually injected. There was marked increase in the rate of venous flow in the kidneys. Blood flow began again in two of the three glomeruli originally open in the right kidney; but in the left kidney the reversed flow in the posterior arteries remained sluggish and there was no glomerular circulation. After another hour, in which no further change occurred, 1 cc. of 30 per cent urea was slowly injected intravenously. This caused a partial cardiac arrest, with complete recovery in about nine minutes. One cubic centimeter of blood was then withdrawn from the abdominal vein. After 3 minutes, during which no blood was flowing in the ureteral vessels or in the posterior renal artery, sudden resumption of flow in these vessels occurred which soon became so rapid that it was difficult to detect the direction of flow. In the right kidney, flow in the posterior renal artery was more rapid than before injection, and rapid circulation became

visible in 5 glomeruli posterior to those in which circulation had originally been seen. This experiment shows that although a saline injection produced an increase in the rate of venous blood flow, it failed to cause reversed flow in the posterior renal artery. When urea was injected blood flow in the collaterals became much more rapid and more glomeruli were opened to circulation.

In two similar experiments aortic and renal portal blood pressures were measured before and after the urea injections. After recovery from temporary partial cardiac arrest, which occurred despite very slow injection, the great increase in rapidity of reversed flow occurred, together with increase in the number of glomeruli in which blood was flowing; aortic pressure, however, was *lower* than before the injection of urea; saline injection, on the other hand, increased the aortic pressure with no effect on the collateral circulation. Urea must, therefore, dilate the small vessels of the ureteral wall.

4. *Effects of ligation of the collateral vessels.* The space between the posterior pole of the kidney and the ureter is covered by a sheet of peritoneum which carries branches of the posterior renal artery which constitute the chief anastomoses with the ureteral vessels (fig. 3). In three experiments these vessels, as well as all the renal arteries, were ligated, urea injected and the frogs kept in an oxygen atmosphere. The results were as follows:

Frog 12. No urine excreted in 48 hours following the operation. Marked edema. Direct observation at the end of 48 hours showed circulation in only 5 visible glomeruli in both kidneys. A small section of the right kidney was receiving blood through a branch of the posterior renal artery connecting with the ureteral vessels at a point anterior to the separation of the ureter from the kidney (fig. 3). There was no hemorrhage during or after the operation.

Frog 13. No urine in 48 hours after operation. Marked edema; weight before operation, 249 grams; 48 hours after, 299 grams. No glomerular circulation visible. Slow reversed circulation in one branch as in frog 12.

Frog 15. Fourteen cubic centimeters of urine in 44 hours. Edema: weight before operation, 198 grams; 44 hours after, 238 grams. The ligature on one artery to the anterior part of the kidney was found to be loose and normal arterial circulation was present in two visible arterial branches in the middle of each kidney.

In a fourth frog only the vessels between the posterior pole of the kidney and the ureter were ligated, the renal arteries remaining open, in order to make sure that the ligatures did not obstruct the ureter. Sixty-seven cubic centimeters of urine were obtained in 46 hours. No edema developed.

These experiments show that when all renal arteries are ligated and, in addition, the collateral circulation which we have described is stopped urine is not excreted in amounts sufficient to be detected by catheterization or examination of the exposed bladder.

5. *Perfusion and injection of ligated kidneys.* Beddard pointed out the

fact, which our experience confirms, that after ligation of the renal arteries the glomerular capillaries become plugged with clotted blood. When, at the end of an experiment, the animal with ligated renal arteries was perfused with saline via the aorta the entire anterior and middle portions of the kidneys were seen to be dotted with infarcted glomeruli and many of the arterioles appeared as red lines. In the posterior part, however, particularly under the fold of peritoneum which covers this region, the arteries and the glomeruli were washed free of blood. But in the animals in which the collateral circulation had been suppressed by additional ligatures, the glomeruli of the posterior pole were infarcted. In these, however, at the level where the ureter begins to be embedded in the kidney tissue there was still a narrow zone in which the glomeruli were washed free of blood during the perfusion. This corresponds to the area whose collateral blood supply could not be ligated as in frog 12, above (fig. 3).

The total number of glomeruli in which circulation is maintained after ligation of the renal arteries cannot of course be estimated by inspection of the ventral surface of the kidney. Estimates based on serial sections however have shown that in one experiment at least 900 glomeruli in the posterior part of each kidney were supplied by the collateral circulation which we have described.

Bainbridge and Beddard decided that in their experiments all glomerular circulation had been abolished because no glomeruli were stained by an aortic perfusion of Berlin blue. We regard this method as unreliable, having found that many glomeruli in the posterior pole of the kidney, from which the blood had been washed out by saline perfusion which reached it via the collateral vessels, were not stained by the subsequent perfusion with Berlin blue. We attribute this to the minuteness of the anastomosing vessels and the viscosity of saturated Berlin blue solutions. The following experiment is illustrative:

Frog 4. All renal arteries ligated; collateral vessels not. Twenty-one hours later, 0.11 cc. urine was taken from bladder. Aortic perfusion with Clark-Ringer cleared all of the vessels (including glomeruli) of the posterior pole of the kidney. Saturated Berlin blue was then perfused at 56 cm. pressure for 35 minutes. Flow into the kidney was scanty, hence after 25 minutes, 1 cc. of 30 per cent urea was injected into the perfusion stream and the posterior pole of the kidney together with the ureter was bathed in 30 per cent urea solution. Perfusion flow increased, more vessels in the wall of the ureter became patent, the posterior renal artery, peripheral to its ligature, distended and the dye solution appeared to pass into the kidney.

Serial sections of the posterior part of the kidney were made and examined. Only 16 per cent of the glomerular sections contained dye; less than 4 per cent contained more than a trace of dye in a single capillary loop.

As a control the vessels of frog 9, in which no renal arteries were ligated, were similarly perfused with saturated Berlin blue solution for 2 minutes only. Serial

sections of the posterior part of the kidney showed that 85 per cent of the glomerular sections contained dye; 74 per cent contained traces of dye in more than one capillary loop or a large amount in a single loop.

These data show that after arterial ligation, the injection of the glomeruli in the posterior pole with dye was far from complete, even though they were clearly accessible to the saline perfusion fluid. Not only were relatively few glomeruli stained, but those which were showed for the most part merely a single fleck of dye in their sections, which might easily have escaped observation if low powers of magnification were used.

In experiments similar to those just cited it was found that fluid gained access to the glomerular capillaries through the efferent vessel. In anterior parts of the kidney, where the glomeruli remained plugged with blood clots following the perfusion, blue dye was seen in the capillary loops between the clot and the endothelium. The afferent vessel was filled with blood and contained no dye: the dye-filled efferent vessel was traced in the serial sections to the capillaries. The injection must have occurred therefore by way of the renal portal system.

I wish to express my deep appreciation to Dr. A. N. Richards for his aid and suggestions during the course of the work and the preparation of the manuscript.

SUMMARY

1. In this series of experiments the observations originally made by Nussbaum on R. esculenta and confirmed by Bainbridge and Beddard for R. temporaria have been confirmed for R. catesbiana. *a*. Ligation of all the renal arteries results in cessation of urine elimination. *b*. Injection of urea after ligation of all the renal arteries results in resumption of urine elimination.

2. Contrary to the belief of those investigators the excretion of urine which follows injection of urea into frogs whose renal arteries are ligated is conditioned upon the establishment of collateral circulation which effectively supplies the glomeruli of the posterior parts of both kidneys. The existence of the collateral circulation was demonstrated by direct observation of rapid reversed blood flow in the main trunk of the posterior renal artery and active circulation in the glomeruli supplied by it, as well as by the observation that the glomeruli of the posterior pole of a kidney whose arteries are ligated can be perfused from the aorta.

3. The collateral circulation is derived from one of the two divisions of the normal arterial supply to the ureter; viz., a branch either of the inferior mesenteric or of the iliac artery. The other division of ureteral supply is the posterior renal artery and this is ligated in the Nussbaum experiment.

4. Under the influence of urea, but not spontaneously or from saline

injection alone, the peripheral ureteral vessels are so dilated that the peripheral distribution of the ligated posterior renal arteries, including its glomeruli, is provided with effective blood supply. As many as 900 glomeruli in the posterior pole of one kidney have been counted as having received blood by this pathway.

5. When the collaterals between the ureter and the kidney as well as all the renal arteries are ligated no urine is excreted even after the injection of urea.

6. Absence of glomerular staining after perfusion with saturated Berlin blue is not trustworthy evidence that glomerular circulation is abolished by ligation of the renal arteries.

7. The elimination of urine after ligation of the renal arteries as described by Nussbaum and Bainbridge and Beddard was not, we believe, the result of tubular activity.

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ELECTRICAL ACTIVITY OF THE CEREBRAL CORTEX IN THE UNANESTHETIZED CAT[1]

MARGARET B. RHEINBERGER AND HERBERT H. JASPER

From the Emma Pendleton Bradley Home and Brown University, Providence, R. I.

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Experiments upon the electrical activity of the higher brain centers in animals maintained under some form of anesthesia and held in a restricting holder have raised the question as to how this activity can be compared to that of the normal unrestrained animal. The present study was undertaken in the attempt to obtain potentials directly from various cortical surfaces while the cat was under dial anesthesia and when it was completely unanesthetized and unrestrained. This included 1, a technique for maintaining electrodes in the skull so that records could be repeated over several days on the same animal; 2, simultaneous recording from four heterologous regions of one hemisphere; 3, comparison of the simultaneous activity from bilaterally homologous areas; 4, controlled conditions of specific and generalized stimulation; 5, a comparison of results from both monopolar and bipolar methods of recording, and 6, complete autopsies with microscopic sections through the regions beneath the electrodes.

TECHNIQUE. *Electrodes.* Fine silver electrodes were used made of wire 0.5 mm. in diameter rounded to a small ball on one end by heating, and insulated except for the extreme end by combined coatings of Duco Household Cement and flexible collodian. Insertion of the electrodes was carried out under surgical dial anesthesia with complete aseptic precautions. Following reflection of the skin and subcutaneous tissues, straight sided burr holds (1.5 mm. diameter) were made through the skull over predetermined cortical areas. Care was taken not to injure the dura. The rounded ends of the silver wires were inserted through the holes to the surface of the dura and secured by sterile wooden wedges which were then cut flush with the skull surface. All wires were led posteriorly and fastened in one or more places to the muscle sheath, connective and subcuticular layers reapproximated over the wires, and the skin sutured with the Halsted subcuticular stitch.

Ten to twelve electrodes could thus be fixed in the skull with the ends of the wires emerging through the skin as far down on the neck as compatible with free head movement. A sterile dressing covered the wound, which healed completely except for that portion of skin through which the wires emerged. Litz wire leads approximately 100 cm. in length were soldered to the ends of the silver wires, bound into a cable, and fastened to a harness and leash so that all of the leads could be plugged in at once and still allow the animal free movement to the extent of the leash. After complete recovery from the dial the cat did not show any annoyance from these attachments.

[1] Research supported by a grant from the Rockefeller Foundation.

At the close of the experimental period (7 to 10 days) the animal was perfused with saline and 10 per cent formalin. Autopsies showed that infection had never spread beyond the immediate vicinity of the open wound. Electrode positions were checked by measurement and by cellophane tracings of the skull, dura, and cortex. Needle punctures through the burr holes served to localize the electrodes for histological study of the brain tissue which gave more precise knowledge of the region, the cell structure, and condition of the cortex. Although in a few placements depressions of the cortex were visible, in no case was there any abnormality of cell structure due to the presence of the electrodes.

Electrode placements. Pairs of electrodes, 2 to 5 mm. apart, were placed on each of four heterologous regions in one hemisphere supplemented by electrodes on bilaterally homologous areas or set unilaterally in varying relationships to the four pairs. No attempt has been made to obtain the outside limits of an area but rather we have restricted ourselves to a comparison between animals of as nearly comparable place-

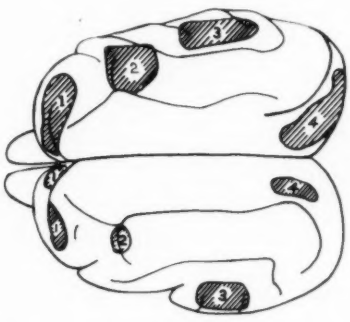

Fig. 1. Composite diagrammatic representation of the cortical areas from which potentials were recorded in seven cats. The regions are taken as representative of 1, the motor; 2, the sensory; 3, the auditory, and 4, the visual areas.

ments as possible. Area 1 has been confined to the sigmoid and coronal gyri, area 2 to the anterior and medial suprasylvian gyri, area 3 to the region bounded by the suprasylvian sulcus, including mainly that between the ectosylvian sulci, and area 4 to the posterior lateral gyrus. For convenience of reference, the terminology of motor, sensory, auditory, and visual areas has been used interchangeably for the designated regions 1, 2, 3 and 4 respectively. Stimulation by condenser discharges of the electrodes in place gave no movement response from any region except area 1, the reaction obtained varying from limb to neck and face movements in the different animals. No effect of direct stimulation of the sensory electrodes was observed even when subsequent autopsy showed them to have been placed slightly anterior to the sulcus ansatus.

Recording techniques and apparatus. All records were taken with the animal resting on a cushioned chair in an electrically shielded, sound proofed room. The controls of all stimuli together with the recording system were placed in adjacent shielded rooms. Auditory stimuli (500 to 2000 cycles at 20 to 80 db. above human threshold), visual stimuli (7 millilamberts direct and flickering light), and tactual stimuli were all signalled on the record as they were administered, the light flicker being recorded with a photo cell. Independent simultaneous recording from four brain regions was possible through four completely independent condenser coupled push-pull amplifiers connected to a four element Westinghouse Type P-A Oscillo-

graph. The over-all sensitivity of the recording system was constant for all frequencies between 1 and 1200 cycles.

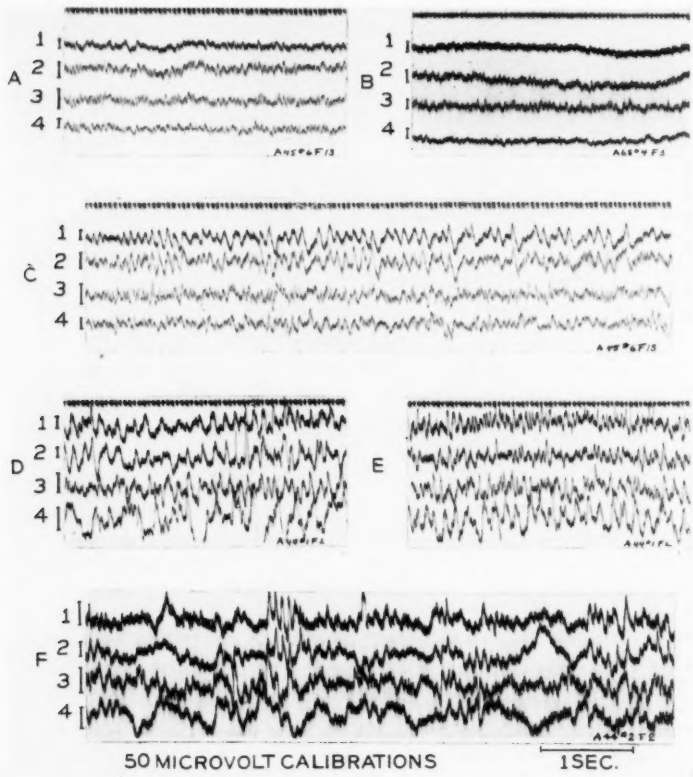

Fig. 2. Variations in states of general activation as represented by cortical electrograms. All records obtained with monopolar leads from 1, the motor; 2, the sensory; 3, the auditory, and 4, the visual areas. Negativity on the cortex is represented by an upward deflection.

A and B: Activation records obtained 1 week postoperatively during moderate (A) and intense (B) excitement (cat box effect, see text). C: Activity obtained with subsequent relaxation of the same animal as in A. D and E: 24 hours after surgical dial anesthesia. E follows D by 90 seconds during which time visual and auditory stimuli were administered. Note the increased frequency in all areas in E. F: Same animal as D and E completely relaxed (asleep) 48 hours following dial.

RESULTS. *Resting activity.*[2] (*a*) *Postoperative anesthesia.* Studies were completed on seven cats. Eighteen to thirty hours postoperatively, when

[2] We have used resting and spontaneous to designate that activity present when no stimulus deliberately introduced may be held immediately responsible for the pattern observed.

the animal was still anesthetized with active reflexes but little spontaneous movement, there was continuous activity at rather constant amplitude, interrupted occasionally by alternating trains of high and low amplitude potentials. The prominent frequencies ranged from 5 to 15 per second with amplitudes of from 60 to 150 microvolts, although frequencies of 3 to 4 per second were not uncommon in the visual area and 20 to 35 per second were also present, most markedly in the auditory region. Superimposed frequencies tended to be multiples of the major rhythms, but there was no simple relationship between the amplitude of a slow frequency and that of its multiples. The outstanding characteristic of the activity was the relative lack of change over periods of several hours if the animal was not deliberately disturbed (fig. 2D).

(*b*) *Unanesthetized animal.* Beginning with the return of spontaneous movement (usually by the second or third postoperative day) the potentials became more variable from moment to moment.[3] Apparently associated specifically with states of complete relaxation or sleep, large slow irregular potential changes, often 100 to 200 microvolts in magnitude, were observed. Periodic interruptions of the slow irregular rhythms by regular waves at a frequency of 12 to 15 per second made this type of cortical activity very similar to that recorded from the human cortex during sleep (fig. 2F) (Jasper, 1936).

Often appearing simultaneously in all regions lower amplitude potentials (30 to 50 microvolts) at frequencies of 20 to 50 per second replaced the slower rhythms for short periods of time unrelated to any known stimulus or visible change in the animal. When persisting for more than a few seconds, however, these more rapid potentials were definitely correlated with increased restlessness or alertness, preparation for movement, or movement itself (Derbyshire et al., 1936; Travis, 1936). A gradual change to and prolonged continuation of this activation pattern[4] (fig. 2 A and B)

[3] Although complete recovery from dial anesthesia could not be said to have occurred at this time, potentials similar to those to be described were recorded during the remainder of the experimental period.

[4] The word activation is used in place of "excitation" previously employed (Jasper, 1936) to denote any stimulating conditions which tend to arouse the animal to activity or increased neuromuscular tension which gives the impression of alertness. This is in conformity with the well founded criticism of Fessard (1936) of the use of excitation state relative to those conditions which affect the rhythmic discharge of isolated neurones. In some of these records associated with moderate degrees of activation of the animal the larger amplitudes, rhythms of for example ten per second, might be replaced by lower amplitude potentials at frequencies of 20, 30 or 40 per second appearing as multiples of the slower resting rhythm. With the most extreme activation the slow rhythms drop out completely and no fast rhythms can be detected other than the slight fuzziness of the line seen superimposed upon the slow potentials previous to activation. This complete blocking of rhythmic electrical response is comparable in all respects to that observed for the occipital alpha rhythm in man in response to visual stimulation. (See also Rempel and Gibbs, 1936.)

while the animal was apparently resting comfortably was so regularly associated with the subsequent need for elimination (often delayed as long as 10 to 15 minutes after the onset of the activation record) that "cat box effect" became a laboratory expression. It provided a striking demonstration of the association of this pattern with internally initiated stimuli of a disturbing or activating nature.

Transitions from the slow random potentials to the activation record were often abrupt. Progressive changes between the two, however, showed periods during which more or less regularly repeated frequencies from 8 to 15 per second (usually with amplitudes of 50–100 microvolts) appeared in all regions (fig. 2C). These potentials resembled the human alpha rhythm in form, especially in the motor and sensory areas, and were less readily differentiated specifically from those observed in the anesthetized animal than were either the activation record or the slower potentials characteristic of sleep. The electrogram was observed only when the animal was known to be awake (eyes partly open) and was easily disturbed by stimuli. It might be considered as representing an intermediate state between the conditions of marked activation and sleep.

Bioelectric and architectonic differentiation. Despite the wide variation in bioelectrical activity which occurred in all regions, under certain conditions distinct electrograms were obtained from different cortical areas. Although we could not readily differentiate between the records from the pre- and postcentral areas, under dial anesthesia and, to a lesser extent, in the resting unanesthetized cat there were distinct differences between the bioelectric patterns of activity from the sensory-motor, sylvian and occipital regions (fig. 2 C, D, E). In the dialyzed animals the regional electrograms seemed to be characteristic and fairly constant although there appeared to be no pattern which was unique to a given area. In the normal resting animal the patterns of the areas were different at a particular instant but later, while there were still differences in the potentials, the pattern previously occurring in one region might be found in a widely separated region. There was no differentiation between areas when the activation pattern was present (fig. 2 A and B). Under all conditions there was more differentiation with bipolar leads.

No attempt has been made to relate such differences to the limits of architectonically distinct areas. The relative constancy of the regional electrograms obtained under dial anesthesia and the essential similarity between bilaterally homologous areas suggest a possible correlation with structure and function of the cortical fields represented (Kornmüller, 1935, 1937). In view of the variations in activity which tend to obscure any regional differences which may exist, especially in the normal animal, a functional differentiation seems as probable as one based upon cytoarchitectonic structure.

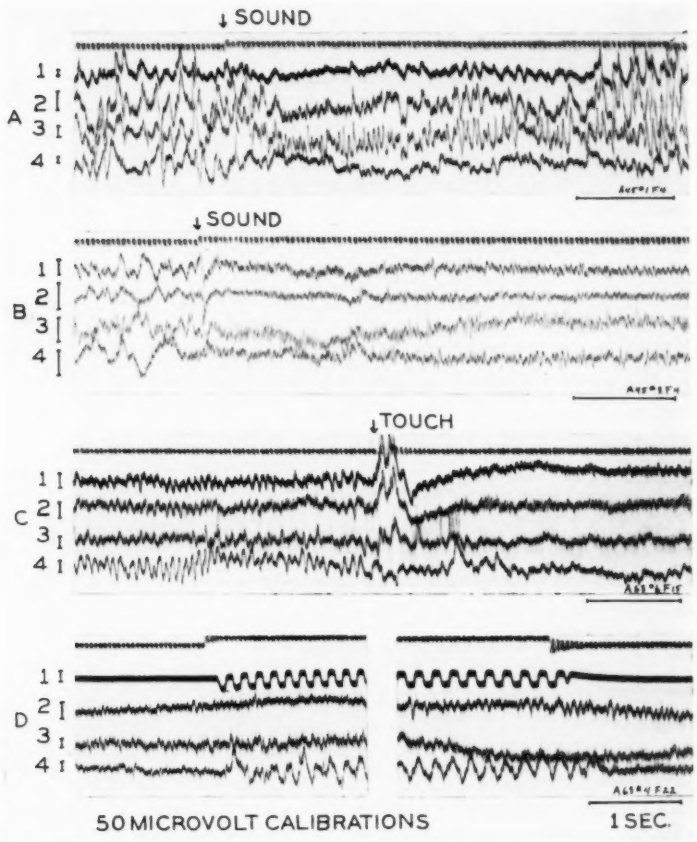

Fig. 3. Effects of stimuli on the electrograms of the anesthetized and unanesthetized cats. A: 24 hours after dial. Sound stimulus causing increased frequency in the auditory region with blocking in the other regions. 1, motor; 2, sensory; 3, auditory; 4, visual areas. Monopolar recording. B: Same animal 9 days postoperatively. Note the similarity of response of all regions to the same intensity of sound as used in A. 1, 2, 3, 4, same. Bipolar recording. C: Tactual stimulus to the left foreleg. 1, left motor; 2, right motor; 3, left sensory; 4, right sensory. Monopolar recording. D: Introduction and cessation of a flickering light stimulus at 5.2 per second. Note the following of the flicker frequency by the potentials of the occipital region. 1, photocell; 2, sensory; 3, auditory; 4, visual areas. C and D same animal, 8 and 6 days postoperatively respectively.

Specific effects of controlled stimuli. (*a*) *Post-operative anesthesia.* In the period 24 hours after dial the effect of a stimulus on existing activity was to 1, block the prestimulation slow frequency potentials, or 2, suddenly

increase the frequency and regularity. The complete block was seldom seen. These effects had a variable latency of approximately 50 to 100 milliseconds and seldom lasted for more than 1 or 2 seconds.

Although regional differences in response were observed no stimulus used affected only one region. For example, a sound stimulus which repeatedly produced a definite increase in frequency and regularity in the potentials from the auditory region greatly decreased the amplitude of the motor and sensory potentials (fig. 3A). Tactual stimulation produced (in some cases) increased frequency and regularity of potentials in the sensory-motor region with depression or little change in the activity of other regions.

With repeated stimulation there often occurred at approximately the same time an increase in the frequencies from all regions (fig. 2E, compare with D). The effect usually lasted for 1 to 2 minutes with a gradual return to the slower rhythms during a period of rest.

(*b*) *Unanesthetized animal.* In the normal unanesthetized animal an effective stimulus produced the activation electrogram (fig. 3 B and C). With the exception of the following of the frequency of a flickering light by the potentials of the occipital area (fig. 3 D)—independent of the activity of the rest of the cortex, only isolated indications of localized responses were observed.[5] The responses had similar latencies but were of longer duration than those observed in the dialized animal. Continued stimulation often produced an activation electrogram lasting for many minutes.

Within the narrow range of variation employed no constant relationship was seen between the absolute intensity of the stimulus and the magnitude of the response. On the other hand there appeared to be a correlation between the extent or spread of cortical response to any stimulus and the degree of activation of the animal. This was observed not only in the comparative effects of the same stimulus on the potentials of the anesthetized and unanesthetized cats but also in the duration of the activation record and the extent of cortical area involved. When the animal responded overtly to the stimulus, i.e., by raising the head or subsequent movement, the high frequency, low amplitude electrogram or the apparent blocking of all rhythmic activity appeared simultaneously in all leads and continued for 10 to 15 seconds or longer. Less obvious disturbance was usually accompanied by a shorter lasting effect and a less complete blocking during which the previous activity of one or more regions might continue unchanged. An animal which at first responded maximally to a given stimulus might subsequently (when very relaxed) show no disturbance, visible or electrical, to the same intensity of stimulation. A similar lack

[5] In one experiment (the series of films recorded on one day) sound stimuli gave rise, after a delay of 0.3 to 3 seconds, to large slow waves almost convulsive in character in the auditory region alone, accompanied by complete blocking of the slow rhythms in the other areas.

of effect was observed from stimuli introduced when an extreme activation record was present. Such variation in the responses of the normal animal suggests that the more transient and localized effects produced in the dialized cat by stimuli of the same absolute intensities may be explained on the basis of less intense activation due to the anesthesia. If such is the case it should be possible, on further investigation, to determine conditions of stimulation under which the cortical potentials of the normal cat will also show differential regional responses.

In general in both the normal and anesthetized animals the occipital area reacted less often in accord with the other regions while the motor and sensory records showed simultaneous response to the majority of stimuli given. This synchronism between the pre- and postcentral areas is in accord with other experimental evidence of the interrelationship of these regions (Dusser de Barenne, 1916).

"Evoked" potentials. "On" and occasional "off" effects were observed in response to stimuli in both the anesthetized and unanesthetized cats. Although the responses varied considerably and at different times from the same regions resembled all of those pictured by Gerard, Marshall and Saul (1936), the most usual form was that of a diphasic or triphasic wave followed by a positive and often prolonged negative after potential. The peak of the first deflection fell usually from 50 to 100 milliseconds after the stimulus, while the total duration of the response might be from 200 to 500 milliseconds or longer. The "off" effect, observed infrequently, was similar to the first part of the "on" response and appeared about 80 to 100 milliseconds after the end of the stimulus. These times are somewhat longer than those obtained by Bartley (1934) for light stimuli. An interpretation of the significance of the slow afterpotentials has been attempted in a previous publication (Jasper, 1936).

Discussion. Our results may not be in fundamental disagreement with those of Kornmüller (1935, 1937) and others who find localized response to afferent stimulation ("Feldaktionsströme") and characteristic patterns of spontaneous resting activity ("Feldeigenströme") referable to definite cytoarchitectonically differentiated cortical areas. If the bioelectric pattern of the cortical surface is determined primarily by the cytoarchitectonic structure immediately beneath one would not expect to find completely distinct types of resting activity from separate areas since the differences in cell structure are more or less statistical in nature, the cytoarchitectonic areas being based upon the predominance and organization of certain types of cells rather than upon completely specific units. This probably explains why the differences in bioelectric activity which can be detected under certain conditions are not clearly distinct patterns composed of elements found only in one region but are due rather to a predominance of a given type of activity and to the sequence of potential rhythms.

It is rather difficult to understand the cytoarchitectonic determination of patterns of bioelectric activity when this activity, under certain conditions, appears exactly the same in all regions, and each region shows variations so great as to overlap and to make quite insignificant any consistent differentiation according to cortical area. These changes appear to be definitely associated with different conditions of activation of the cortex due to specific or generalized excitation. Since different excitatory conditions as well as different cytoarchitectonic structures must certainly exist within the cortex, we do not feel that it is possible at present to evaluate properly which is more important in determining the pattern of spontaneous bioelectric activity.

The fact that specific forms of electrical response to a given stimulus may be, under certain conditions, very definitely localized while changes in the electrical activity of all regions are observed in response to any afferent stimulus which is sufficiently disturbing to the animal illustrates in a striking manner both the localized and mass functions of the cortex (Lashley, 1933; Gerard, Marshall and Saul, 1936).

All cats showed slow rhythms which disappeared under certain conditions of activation. This suggests that the absence of slow rhythms in some human subjects may be due to similar or analogous functional conditions. The tendency for the slow rhythms to be replaced by low amplitude potentials at frequencies which are multiples of those characteristic of the slow rhythms indicates a desynchronization process. However, the complete absence of detectable electrical activity, except for the slow potential swings (called "after-potentials" above), immediately following intense afferent stimuli suggests genuine arrest, temporarily, of the rhythmic activity of the cortical neurones since it does not appear probable that such a complete absence of the more rapid discharges together with the slow potential swings could be due to changes in synchronization. There may be a depression of activity or "inhibition" during the slow positivity as has been shown by Eccles (1935) for the cervical ganglion and by Gasser (1935) for the spontaneously firing phrenic nerve. There may also be a blocking of rhythmic activity ("inhibition" of the Wedensky type) during the slow negativity (Jasper, 1936). These changes which control to some extent the rhythmic discharges of the individual neurones may be basic to their change in synchronization with adjacent cells.

The wide spread of activation from one cortical region to another, probably involving both cortical and subcortical conduction pathways, differs from the narrow spread found by Adrian (1936) due in some degree to lack of anesthesia but primarily to the fact that we were dealing with sustained effects of afferent stimulation rather than to immediate effects of direct electrical stimulation. Our results favor Adrian's interpretation of cortical potentials as related to rhythmically firing central neurones of long

time characteristics, their activity depending to a large extent upon local excitatory conditions and not directly upon the integrity of self-exciting neurone chains (Lorente de Nó, 1933; Bishop and O'Leary, 1936).

SUMMARY

Small silver electrodes were fixed in the skull so as to make contact on the dura over the motor, sensory, auditory, and visual cortical areas. Flexible leads were brought out through the skin in such a manner as to permit the animals to recover completely from the operation and engage in normal unrestrained activities while at the same time records could be taken directly from the cortex over a period of several days. Histological studies of the brain regions over which the electrodes were placed served to control the accuracy of localization and histological structure of the areas used as well as to show that records were taken from normal brain tissue.

The results from simultaneous records of the activity of four different cortical areas with both monopolar and bipolar recording may be summarized as follows:

1. The pattern of cortical activity in the partially dialized and in the normal undisturbed cat was characterized by more or less regular rhythmic potential changes at frequencies from 3 to 15 per second.

2. Bilaterally homologous areas showed essentially similar bioelectrical activity.

3. Some fairly consistent differences in pattern of activity from the anesthetized animal could be identified with different cortical fields, but the variations in activity of each region in the normal cat were so great that bioelectric differentiation appears to be primarily related to differential states of activation rather than to specific cytoarchitectonic structure.

4. The greatest similarity of resting activity and response to afferent stimulation was found between the sensory and motor areas.

5. The electrogram from all regions was characterized by low amplitude higher frequency potentials when the animal showed behavioral indications of being generally aroused or activated due either to controlled external stimuli or internal organic states. Relaxation was associated with a return of the slower rhythms while the pattern characteristic of sleep was similar to that described for man during sleep.

6. The electrical response of the cortex to afferent stimulation included "on" and "off" effects and slow positive and negative after-potentials associated with depressions of rhythmic activity as well as changes in frequency and amplitude of the spontaneous potentials.

7. Changes in the electrical activity of all regions of the cortex usually followed a single effective afferent stimulus although the changes in a

given region could be more intense and of a different form than those in other regions.

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THE EFFECT OF HEAT ON BLOOD AND LYMPH FLOW FROM THE GASTRO-INTESTINAL TRACT[1]

J. M. BEAZELL, C. R. SCHMIDT AND A. C. IVY

From Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois

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Direct quantitative studies on the effect of heat on blood flow in the intestine have never been recorded. The fact that heat locally applied to a part increases the rate of blood flow has been established chiefly by qualitative and indirect quantitative observations. It has been observed by direct measurement that heat applied externally to a limb of an animal increases the blood flow through the femoral vein (1). In this study we have directly measured the effect of heat applied to the lumen of the small intestine and colon on the rate of flow of blood from the treated segment. In addition, the effect of this treatment on lymph flow and the production of succus entericus has been determined.

METHODS. Heat was applied at various levels of the gastro-intestinal tract of dogs by means of an Elliott Treatment Regulator. In essence this apparatus consists of a thermostatically controlled water bath and an electrically driven pump which circulates the water through a tubular, soft gum rubber, slightly distensible applicator. The applicators are interchangeable so that the size and shape can be varied to conform with the organ to be treated.

Experiments on blood flow. All of the experiments were performed on heparinized dogs under pento-barbital anesthesia. Ten dogs were used to determine the effect of heat on the blood flow from an isolated loop of small intestine and eight were used to make similar measurements on the colon.

The procedure was as follows: a femoral vein was cannulated and connected to a paraffin-lined funnel by means of an oiled rubber tube. This was to serve as a means for returning blood to the animal. A loop of small intestine or colon was exteriorized and then isolated so that all of the blood from the part to which heat was to be applied returned by way of a single vein. In the case of the small intestine this was accomplished by choosing a segment of such length that it was drained by one

[1] This investigation has been aided by a grant from the Council of Physical Therapy of the American Medical Association.

vein. In the colon studies all venous channels except the inferior mesenteric vein were ligated. A drainage tube for the collection of intestinal juice and a thermometer for ascertaining the temperature of the intestinal fluid were purse-stringed into the lumen at the distal end of the segment and the cylindrical rubber applicator, filled with tepid (37.7°C.) water, was inserted and secured at the proximal end. The applicator was of such size that it snugly filled the lumen of the segment. Heparin (10–15 mgm. per kilo) was then injected into the systemic circulation and the vein draining the segment was cannulated. Additional heparin was given every thirty minutes to insure against coagulation. The cannula was connected by means of an oiled rubber tube with a graduated paraffin-lined cylindrical collecting tube equipped with inlet and outlet side arms. A cannula with a lumen comparable to that of the distended vein was used. This step in the procedure was carried out with sufficient rapidity to avoid significant blood loss and venous congestion.

The blood was permitted to flow into the collecting tube, rapidly drained into a paraffin-lined beaker, and returned to the dog by way of the funnel attached to the femoral vein. It was thus possible to maintain a continuous flow of blood with no significant decrease in the circulating blood volume. The collecting tube was placed at a slightly lower level than the segment of intestine to minimize the resistance to the flow of blood. This hydrostatic effect was constant in all experiments. The intestine was kept moist and at body temperature. Under these circumstances the isolated segment of intestine maintained its normal healthy appearance for the duration of the experiment.

The volume of blood collected varied from 30 to 50 cc. depending on the size of the dog. The rate of flow was determined by measuring with a stop watch the time required for each collection. The total volume of blood collected during each 15 minute period was also recorded. Collections were continued and measured in this manner throughout the experiment.

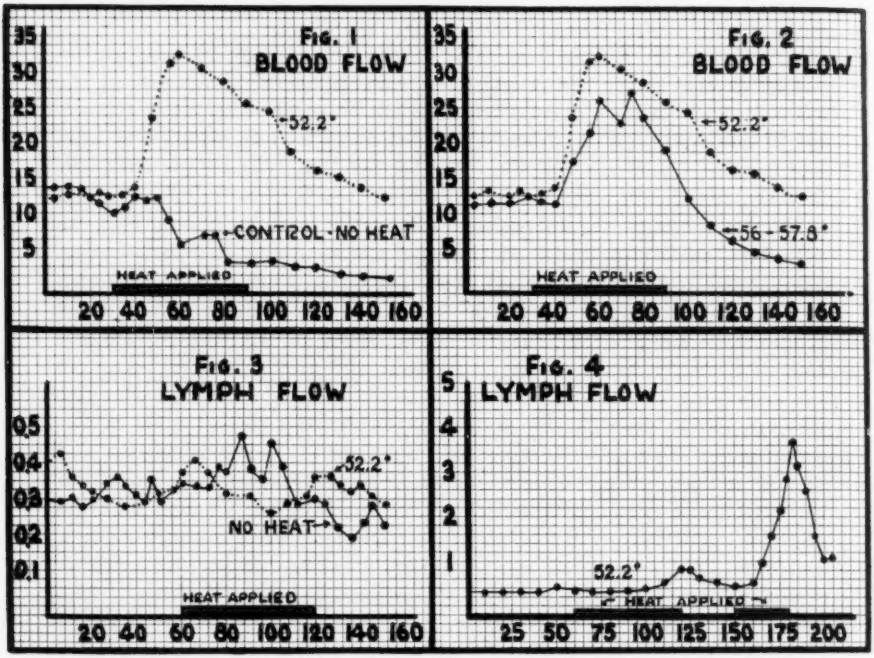
The rate of flow was determined during a control period lasting for 30 minutes or longer. After establishing the basal flow the heat treatment was started. The water was circulated through the applicator at an initial temperature of 46.6°C. During a period of about 15 minutes the water was raised to 52.2°C. and maintained for the duration of an hour. Other experiments were performed with the water temperature at 54.4°, 55.5°, and 57.7°C. Following the period of application of heat, collections were continued for 1 hour to determine the post-treatment rate of flow. In 3 control experiments continuous collections were made without the application of heat, but with the applicator in place. In two instances the heat treatment was extended to 1.25 and 2.25 hours. The effect of a second heat treatment following the 1 hour post-heat interval

was also investigated. The rectal or vaginal, and subcutaneous temperatures were noted at intervals during the experiments.

Experiments on lymph flow. The results obtained on 21 dogs are included in this series. In 4 dogs heat was applied to the lumen of the stomach and lymph was collected from the thoracic duct. The remaining 17 dogs were used to determine the effect of heat on lymph flow from the lacteals. *a.* To study the effect of heat on lymph flow from the lacteals, five hours after a small fat meal the dogs were anesthetized. A loop of jejunum was exteriorized through a midline incision and the cylindrical rubber applicator was inserted and secured with a purse-string. The lacteal draining the segment was cannulated with a small glass cannula having a lumen comparable to that of the lacteal. The rate of lymph flow was followed for 2.5 hours. In 7 of the dogs continuous collections of lymph were made without the application of heat. In the remaining 10 the effect of heat was determined. The heated water was circulated through the applicator during the second hour of the experiment. The water temperature was 52.2°C. in 9 experiments and 57.7°C. in one. In one experiment the heat was reapplied at the end of the 30 minute post-heat interval and maintained for an additional 30 minutes. *b.* To study the effect of heat applied to the stomach on thoracic duct flow, a round balloon applicator 7 cm. in diameter was secured in the lumen of the stomach with a purse-string suture in 4 dogs. The thoracic duct was cannulated in the supraclavicular region and the rate of lymph flow was measured over a 2.5 hour period. The heat was applied throughout the second hour of the experiment at a temperature of 52.2°C.

RESULTS. *Experiments on blood flow.* As a rule the basal rate of flow remained fairly uniform during the control period. In the control experiments in which no heat was applied the rate of flow gradually decreased over the 2.5 hour period. This is illustrated by the composite curve of the results obtained on 3 dogs shown in figure 1. In the same figure is shown the composite curve of the results obtained on seven dogs treated with the temperature of the water in the applicator at 52.2°C. The increase in blood flow resulting from the application of heat is evident.

The results in table 1 show the extent of individual variation that occurred in the case of the small intestine. The results on the colon are also shown, but are averaged because the individual variation in response was small. Regardless of the individual variations, the application of heat always produced a sustained increase in blood flow that lasted throughout the heat treatment and well into the post-heat period. The maximum increase occurred during the last 30 minutes of the heat period. In some experiments the blood flow was approximately quadrupled and in others it was approximately doubled. It should be pointed



Abscissae: Figs. 1, 2, 3 and 4—time in minutes.
Ordinates: Figs. 1 and 2—volume of blood flow in cubic centimeter per minute.
Figs. 3 and 4—volume of lymph flow in cubic centimeter per hour.

TABLE 1

*Effect of heat on blood flow from the small intestine and colon of the dog**

HEAT APPLIED TO	DETERMINATIONS	CONTROL (BASAL) FLOW IN CC./MIN.	HEAT PERIOD								POST-HEAT PERIOD							
			0-15 m.		15-30		30-45		45-60		0-15 m.		15-30		30-45		45-60	
			cc/minute	% of basal	cc/minute	% of basal	cc/minute	% of basal	cc/minute	% of basal	cc/minute	% of basal	cc/minute	% of basal	cc/minute	% of basal	cc/minute	% of basal
Small intestine.	7	12.8	13.8	108	24.6	192	27.4	214	26.7	208	21.8	170	19.5	152	17.8	139	17.0	133
Maximum.		7.4	10.0	135	22.0	297	28.0	378	30.0	405	26.6	360	17.4	235	12.0	162	10.7	145
Minimum.		16.0	17.3	108	27.6	141	29.4	184	29.4	184	29.4	184	24.0	150	22.9	143	16.0	100
Colon	3	19.6	18.0	92	31.5	161	32.4	165	35.8	183	36.4	186	24.5	125	22.7	116	21.2	108

* Temperature of the water in the applicator 52.2°C.

out that the values in the table do not take into account the gradual decrease in the rate of flow that would occur without the application of heat. When this decrease is considered, the increased blood flow produced by the heat is more sustained than the values in the table would indicate. This is evident when the curves in figure 1 are inspected. In the animals reported in table 1 and figure 1, the treated segment was grossly normal at necropsy. Microscopically it was found that small scattered areas of mucosa were denuded of epithelium and erythrocytes were present in the tissue spaces.

In heat treatments of more than 1 hour duration (52.2°C.) the maximum effect on blood flow was obtained during the first 45 minutes; the effect was then sustained throughout the remainder of the treatment. Even when the heat was applied for 2.25 hours no grossly demonstrable injury was present in the segment. When the heat was reapplied at the end of the 1 hour post-heat period, a second increase in blood flow comparable to the first was obtained.

Body temperatures taken rectally or vaginally, and subcutaneously during the course of the heat treatment, failed to show any consistent change. The body temperature sometimes rose or fell slightly indicating that our experimental procedure was not deleteriously influencing the results. This should be expected, since on transferring the blood from the mesenteric to the femoral vein some cooling of the blood occurred.

The temperature of the mucosa of the segment was not directly recorded. However, the thermometer in the lumen of the segment recorded the temperature of the intestinal juice. The temperature of the intestinal juice, when the temperature of the applicator was 52.2°C., was never more than 1.6°C. higher than the rectal temperature. The highest temperature recorded in the intestinal juice was 39.5°C. This indicated that the heat of the applicator in contact with the mucosa was rapidly dissipated by the increased blood flow in these experiments. Without doubt the temperature of the cells in the mucosa and of the blood in the villi was elevated considerably more than 1.6°C.

Abnormal effects. In one experiment on the intestine, in which the temperature of the water in the applicator was held at 52.2°C., the applicator chosen was of sufficient size to distend the intestinal segment slightly. In this experiment the intestinal fluid became blood tinged and the mucosa was grossly damaged at post-mortem. In three other experiments on the intestine the temperature of the applicator was raised to 54.4°, 55.5°, and 57.8°C. In these experiments the increase in blood flow was less than observed at 52.2°C. (fig. 2). In the first two experiments the intestine was grossly injured. In the latter (57.8°C.) the intestine was not grossly injured. This was ascribed to the fact that the intestinal segment was not "rounded out" by the applicator. Because

of the muscular response of the colon segment to the irritation of the applicator, difficulty was experienced in selecting an applicator that did not produce injury at a temperature of 52.2°C. In five experiments on the colon injury occurred, and, as in the case of the intestine, the increase in blood flow was not as marked as when no injury resulted. In fact, after about 45 minutes the blood flow decreased below the control rate. Thus, if the applicator at a temperature of 52.2°C. mechanically distended the segment or even caused the colonic or intestinal segment to contract vigorously, injury of the mucosa resulted and the vascular response was abnormal. Obviously this method does not provide data indicating the exact temperature at which the intestinal or colonic mucosa is damaged, a question that does not immediately concern the problem under investigation.

The *secretion of succus entericus* was markedly stimulated by the heat in all experiments except one. Average values obtained in the seven uncomplicated experiments on the small intestine were as follows: control (preheat) period, 0.7 cc. per hour; one hour heat period, 10.8 cc. per hour; post-heat period, 10.0 cc. per hour.

Experiments on lymph flow. The effect of heat on lymph flow from the lacteals was determined by comparing the rate of flow in 7 untreated dogs with that in 8 dogs treated at 52.2°C. and in which gross injury did not occur. Composite curves of the results obtained in each series are shown in figure 3. It is apparent that a temperature of 52.2°C. has no significant effect on the volume of lymph flowing from the treated segment of intestine. The curve shown in figure 3 indicates a slight decrease in the rate of flow during the heat period. However, since in some of the experiments there was a correspondingly slight rise, we feel that the change was insignificant.

The total volume of lymph collected during the 2.5 hour experimental period varied from 0.25 cc. to 5.5 cc. The marked variation would be anticipated, since there is no uniformity in the size of lacteals draining corresponding segments of intestine in different animals. There was no definite relationship between the size of the dogs and the size of the lacteals. Some of the larger volumes of lymph were collected from relatively small animals. In all of the animals a periodic variation in the rate of flow was observed. No increase in lymph flow occurred when the temperature of the applicator was 46.6°C.

Increasing the temperature of the water to 57.7°C. in one dog also resulted in no change in lymph flow. At autopsy the intestinal mucosa of this dog was grossly normal.

An experiment was performed in which an applicator was used which slightly distended the intestinal segment. The temperature of the applicator was 52.2°C. An increase in the rate of lymph flow occurred at

the end of 42 minutes of heat treatment; this persisted throughout the remainder of the experiment. When the rate of flow increased, the lymph became clearer. An additional 30 minutes of heat treatment was instituted at the end of the post-heat period. The rate of flow increased still further and the lymph became blood tinged, thus definitely establishing the presence of tissue damage. At necropsy the intestine of this dog was found to be grossly injured. The results obtained in this experiment are shown in figure 4.

Heat (52.2°C.) applied to the lumen of the stomach failed to modify the rate of flow of thoracic duct lymph in the 4 dogs studied. The temperature of the applicator was 52.2°C. In all of the dogs the rate of flow tended to fluctuate, but there was no correlation between the fluctuations and the application of heat. During the first hour, the flow for the 4 dogs averaged 0.271 cc. per minute; during the second hour, it averaged 0.266 cc. per minute; and during the last thirty minutes, it averaged 0.280 cc. per minute.

Discussion. The phase of this investigation concerned with blood flow requires little discussion. It is generally agreed that locally applied heat induces an active hyperemia. Therefore the observed circulatory effects were qualitatively predictable. However the magnitude of the response could not be anticipated. These results have established that the increased blood flow produced by the application of heat to the intestine or colon is of appreciable magnitude. This probably accounts for the apparent relatively great heat tolerance of the gastro-intestinal mucosa. Temperatures that would be destructive to the skin were applied to mucous membranes, in this study, with no ill effects, unless some factor was present which interfered with venous return. The markedly augmented blood flow, coupled with an initial abundant blood supply, could readily dissipate the heat and thus prevent an appreciable elevation in tissue temperature. This is borne out by the fact that in no instance was the temperature of the intestinal secretion within the lumen of the treated segment raised more than 1.6°C., in which experiment the initial temperature of the juice in the segment was 37.9°C.

However, if the applicator distended the colonic or intestinal segment, or if the segment contracted forcibly upon the applicator, an applicator temperature of 52.2°C. produced gross injury of the mucosa. Again, if the applicator was relatively small, even an applicator temperature of 57.8°C. in one instance failed to cause gross injury. This indicates the important rôle that the blood flow plays in the dissipation of the heat. If venous return were reduced by distention or contraction of the segment, the the heat of the applicator (52.2°C.) was adequate to cause injury. The rôle that blood flow plays in dissipating heat applied to the skin has been pointed out by Bazett (6). However, the exact temperature at

which the proteins of the cells of the intestinal and colonic mucosa are coagulated remains to be determined.

From a practical viewpoint our results show that if proper precautions, both as to the applicator size and shape and as to the contractile response of the viscus to be heated are observed, the temperature in the applicator may be safely raised to 52.2°C. without causing gross injury to the colonic and intestinal mucosa. Higher applicator temperatures are not safe.

The effect of heat applied to the parietes on lymph flow has been studied by a number of investigators. In 1894 Starling (2) noted that when a dog's foot was kept in water at 60°C. for 5 minutes the rate of flow of lymph was increased. Field, Drinker, and White (3) found that a significant increase in lymph flow does not occur until the temperature of the water into which a dog's foot is submerged is raised to 50°C. Indirect evidence accumulated by other investigators supports this observation. As a result Drinker and Field (4) have concluded that an active increase in lymph flow does not occur until the increased capillary permeability produced by heat has become a pathologic process. This contention is supported by the results obtained in this study. The only experiment in which an augmented lymph flow was noted was the one in which injury was produced.

It is of interest that the increase in the secretion of succus entericus coincidental with the increase in blood flow was not accompanied by an increased lymph flow. This is similar to the observations on the submaxillary glands (5).

SUMMARY AND CONCLUSIONS

Heat was applied to the lumen of isolated segments of the small intestine and the colon at temperatures ranging from 52.2° to 57.7°C. by using a specially devised hollow rubber applicator. Under these circumstances the rate of flow of blood through the treated tissue was markedly augmented, unless the mucosa was injured. In some experiments the rate of flow was doubled and in others it was quadrupled. This procedure had no measurable effect on the rate of flow of lacteal lymph. However, the secretion of succus entericus was markedly augmented.

Temperatures of 52.2°C. were applied to the lumen of the stomach without effecting the flow of lymph from the thoracic duct.

Burns occurred in 3 out of 4 experiments in which the applicator temperature was 54.4°C. or more. When the temperature was maintained at 52.2°C., burns occurred only when the applicator was of such size that it distended the segment, or caused it to contract vigorously. The contractile response of the colon rendered it more sensitive than the small intestine to the injury produced by the heat of the relatively too large

applicators. Injury resulted in a decrease in blood flow and an increase in lymph flow.

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THE RELATION BETWEEN SYSTEMIC AND PULMONARY BLOOD PRESSURES IN THE FETUS

W. F. HAMILTON, R. A. WOODBURY AND E. B. WOODS[1]

From the Department of Physiology and Pharmacology and the Department of Obstetrics and Gynecology, University of Georgia School of Medicine, Augusta

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The establishment of the adult circulation involves the functional separation of the systemic and pulmonary circulation and the obliteration of the placental circulation. Our understanding of these changes is based largely upon our interpretation of structural relationships. Experimental data are deficient.

From the fact that the ductus arteriosus shunts the blood between the two circuits it has been assumed that the systolic pressures are very similar in the two ventricles. The only experimental verification of this conjecture is that of Pohlman (1) who inserted similar pipettes into the two ventricles and observed that the two pipettes filled at similar rates.

Concerning the relative volumes of the pulmonary, systemic, and placental streams, the evidence is contradictory. Arguing from the relative sizes of the lumina of the respective arteries, these three streams are said to be nearly equal (2). The experimental evidence, however, demonstrates that the fetal pulmonary stream is much smaller than the systemic. Pohlman (1), and later Kellogg (3), injected starch granules into the venae cavae and found them in equal numbers in the blood of the two ventricles. Obviously the left ventricular blood was not diluted by a large pulmonary stream.

"To conceive of a capacious but virtually unused vascular plexus developing in the growing lungs—ready at the instant of birth to receive a suddenly re-routed current of blood carried to the lungs for oxygenation" (2)—may well smack of teleology. Teleological also would appear the substitute notion (2) that the fetal lungs receive a stream of blood large enough to measure up to the size of their vessels and incidentally to support respiration after birth when so large a stream would seem to be out of all proportion to the metabolic needs of the lungs. Indeed it is hard to escape, in this field, that which has an "odor of teleology." One thing seems certain—the word should not be used as an epithet for hypotheses we are prematurely discarding.

[1] Now with the State Board of Health, Jacksonville, Florida.

We are here presenting data as to pressure relationships which will put us in a position to evaluate the anatomical data from a physiological viewpoint.

Apparatus and procedure. Our observations were made by the "hypodermic manometer" described elsewhere (4). It gives an accurate photographic record of pressure pulses in cavities or vessels within the undamaged body. These cavities or vessels can easily be entered by a small hypodermic needle connected to the manometer and as soon as entry is made the pressure within registers itself, even to its most rapid fluctuations, upon a moving photographic surface.

With two of these manometers we attempted to record simultaneously the pressures in the right and left ventricles of fetuses.

The experimental animals were pregnant dogs or rabbits as near term as possible. They were anesthetized with ether and morphine, neonal or amytal. The fetuses were delivered through the abdominal wall. In case of the rabbits the placentae slipped off during delivery but the dogs' placental attachment could be maintained, often, throughout the whole procedure.

As soon as the membranes had been stripped off the fetus was placed upon its back on a small board firmly held immediately above the maternal incision. The fore and hind legs of the fetus were fixed to the board with strong rubber bands and the two manometer needles were inserted through the chest wall and into the right and left ventricles. Autopsies were performed to determine that the needles had entered the cavities intended. Where this had not occurred the records were discarded. Calibrations against a mercury manometer showed that the pressures could be read to within 2 or 3 mm. Hg.

Blood pressure relationships before breathing. In figures 1, 2 and 3 are seen several curves from dog and rabbit fetuses. Pressures in the premature fetuses are lower than in those that are at term. Systolic pressures in the right ventricle are about as high as those in the left ventricle. Blood therefore may be passing from the ductus arteriosus to the aorta at each heart beat. The diastolic pressure in the right auricle is the same as or higher than in the left auricle. Caval blood then fills the right ventricle and a mixture of caval and pulmonary blood fills the left ventricle. Pressure relationships are such that, as the ventricle fills, the foramen ovale is not closed and there may be a stream of some force passing from right to left.

Blood pressure relationships while clamping the umbilical cord. In figures 6 and 7 are similar tracings taken during the clamping of the dog's cord. In these observations and many others there was no alteration of the even course of the intraventricular pressure pulsations. Either the flow had been shut off by previous constriction of the umbilical artery or

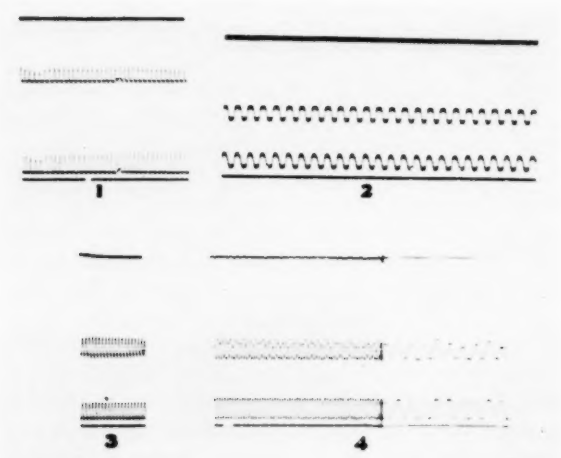

Fig. 1. Ventricular pressures in premature rabbit fetus. Upper curve respiration (respiration has not started). Second curve right intraventricular pressure. Third curve left intraventricular pressure. Fourth curve base line and time every second. Heart rate 56 beats per minute. Blood pressure in millimeters mercury, right, 14 systolic; 0 diastolic; left, 14 systolic, 0 diastolic. (R. 14/0, L. 14/0.)

Fig. 2. Ventricular pressures in dog fetus at term. Curves as in figure 1. Respiration has not started. Blood pressures, R. 50/0, L. 50/0; heart rate 240.

Fig. 3. Ventricular pressures in rabbit fetus at term. Curves as in figure 1. Respiration has not started. Blood pressures, R. 21/0, L. 20/1; heart rate 180.

Fig. 4. Ventricular pressures in same fetus after breathing has started. Curves as in figure 1. Blood pressures at end of inspiration, R. 16/1, L. 28/0; at end of expiration, R. 24/3, L. 30/3; heart rate 240. Average of several respiratory cycles.

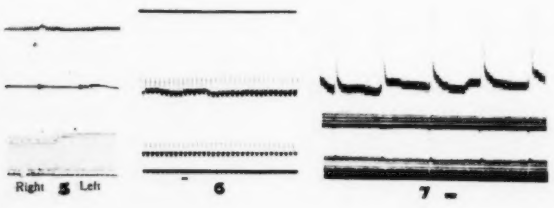

Fig. 5. Ventricular pressures in rabbit fetus after breathing has started. In part of the record the needle point was in the right ventricle and in part it was pushed through the septum and into the left. Confirmed at autopsy. Blood pressures at end of inspiration, R. 26/–1, L. 33/–1; at end of expiration, R. 34/1, L. 36/0.

Fig. 6. Ventricular pressures in dog fetus. Curves as in figure 1. Short mark below indicates clamping cord. Heart rate, 60; blood pressure, R. 45/4, L. 45/2.

Fig. 7. Same after respiratory attempts have started. Heart rate, 240; blood pressure, R. 50/0, L. 50/0.

the simultaneous shutting off of arterial and venous streams when the cord was clamped left the net cardiac work unchanged. We rather think that in spite of precautions and all possible speed we did not successfully shield the cord from such stimuli (e.g., cold) as normally shut off its arterial flow (5). More careful experiments are in order.

Blood pressure relationships during early breathing. The heart and intrathoracic blood vessels including the pulmonary capillaries are to be regarded as elastic containers surrounding fluid which is essentially incompressible and inexpansible. Consequently every change in pressure which occurs in the thoracic cavity will be immediately transmitted to the blood within the thorax. This means that during the first inspiration where the lungs are stretched and the intrathoracic pressure is reduced the pressure within each ventricle is directly reduced by the same amount. The pressure goes up and down during the heart cycle but at a lower level. As soon as the respiration is over the level returns more nearly to its original value.

These periodic fluctuations in pressure affect the peripheral resistance of the two circuits to a different degree. The capillaries and arterioles of the systemic circuit are in the main unaffected by changes in intrathoracic pressure while those of the lung are pulled by the lower intrathoracic pressure away from their contents giving the blood a freer way through them. While the lungs are collapsed the right ventricle has to work against atmospheric pressure and the viscous resistance of the solid lung. After the lung is expanded the heart works against a pressure that is less than atmospheric (somewhere between atmospheric pressure and intrathoracic) and against the elastic resistance of the capillary walls backed up by alveolar air rather than by the relatively solid pulmonary tissue of the collapsed lung.

Thus we would argue that the lowering of the intrathoracic pressure lowers pressure in both ventricles *directly* and in addition further lowers the pressure in the right ventricle *indirectly* by changing the peripheral resistance. (For references see 6, p. 644.)

Our records indicate that this conception holds true. From figures 4 and 5 it is seen that the diastolic pressures in both ventricles and the left systolic pressures drop about the same amount on inspiration. This is due to the direct effect of lowered intrathoracic pressure. The systolic pressure in the right ventricle, however, drops during inspiration three or four times as far. This is due to the indirect factor mentioned above. These conclusions are confirmed by records from many other fetuses which are very similar to those in figures 4 and 5.

The fact that inspiration lowers the systolic pressure in the arteries of the lesser circulation to a greater extent than it does the pressure in the aorta requires explanation. If the ductus arteriosus were open to flow

from the aorta to the pulmonary artery pressures in the two would be equal. In dogs and rabbits, at least, the ductus arteriosus is valve-like. There is a pair of flaps (rabbit) or a single flap (dog) which are competent to stop the back flow of perfused saline from aorta to ductus but allow its free flow from ductus to aorta. Sketches of these structures are seen in figures 9 and 10 as compared with an ordinary arterial branching, figure 8.

Our records testify to the competence in life of this valve-like structure in the dog and rabbit fetus. The ductus can pass blood freely from right to left, but as is true with the foramen ovale, cannot pass it from left to right. These two valves then trap blood in the systemic circuit until the pressure gradient between the systemic and pulmonary circuit keeps them

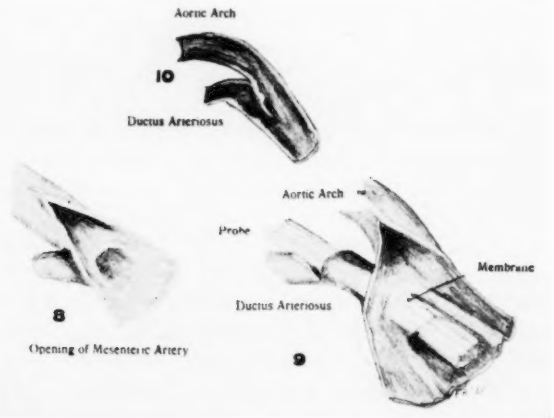

Fig. 8. Opening of mesenteric artery from aorta in full term dog fetus.

Fig. 9. Opening of ductus arteriosus into aorta in full term dog fetus. Note valve-like membrane guarding opening.

Fig. 10. Longitudinal section through opening of ductus arteriosus into aorta in full term rabbit fetus. One of the two valve-like flaps is shown.

permanently closed. After they have been functionally closed for a while adhesions form and the closures usually become "anatomical."

Relationships in the newborn. The newborn rabbit, figure 11, that has breathed successfully for a few hours, has a systemic arterial (left ventricular systolic) pressure which is maintained well above the pulmonary arterial pressure (right ventricular systolic). A permanently negative intrathoracic pressure has apparently been established and the systemic peripheral vasomotor tone has been augmented. The pressures in the two auricles as indicated by the diastolic pressures are about equal and the foramen ovale is probably functioning occasionally.

Relationships in the two-day rabbit. In the two day old rabbit illus-

trated in figure 12 the left auricular pressure has come well above the right. We suggest that this is because the left heart is responding to its increased work, according to Starling's law, with increased dilatation. The increased dilatation is consequent upon increased filling pressure and this in turn serves to close the foramen. It will probably remain closed until adherent. Later hypertrophy of the left ventricle will eliminate the necessity for dilatation and increased left filling pressure so that the adult relationship of similar auricular pressures in right and left heart will be attained. Intraventricular pressures in the unoperated adult range rather widely around the following figures: rabbit, L. 140/0—R. 25/0; dog, L. 180/0—R. 35/0.

It should be emphasized that in accordance with modern conceptions of cardiac physiology a ventricle will hypertrophy if it works harder. How-

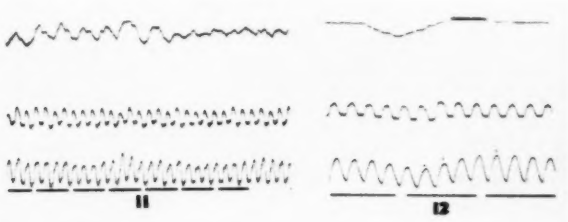

Fig. 11. Ventricular pressures in newborn rabbit. Curves as in figure 1. Blood pressures (average) at the end of inspiration, R. 20/1, L. 30/1; at the end of expiration, R. 23/1, L. 40/1; heart rate, 220.

Fig. 12. Ventricular pressures in two-day-old rabbit. Curves as in figure 1. Blood pressures at the end of inspiration, R. 18/0, L. 27/2; at end of expiration, R. 20/4, L. 47/11; heart rate 270.

ever, it will work harder only if it is filled fuller either by residual blood left from previous incomplete contractions against a heavy load or by increased venous inflow. An increased filling requires an increased filling pressure. Thus it is difficult to explain even the beginning of hypertrophy of the left heart as compared to the right without postulating different filling pressures to the two hearts, and consequently an early functional closure of the foramen ovale.

Conditions in the human fetus are different in that blood may flow either way in the ductus arteriosus. There are no valve-like flaps to guard the aortic opening of the human ductus. Perfusion of the aorta with saline under physiological pressures, resulted in a divided stream. The larger volume issued from the descending aorta and a much smaller volume flowed back through the ductus arteriosus.

Lowered pulmonary resistance, upon the establishment of a negative

intrathoracic pressure must necessarily cause the ductus stream to flow from aorta to pulmonary artery. What causes the stream to stop and the human ductus to close appears to be an unanswered question. It cannot be merely an inherent tendency on the part of the developing ductus to close against hydrostatic forces because, if the hydrostatic forces are large enough as in congenital stenosis of the aortic or pulmonary ring, the ductus may remain patent throughout life. On the other hand, it cannot be a gradual diminution of the difference in hydrostatic pressure at the two ends of the ductus because systemic and pulmonary pressures in all probability gradually grow apart as the chest expands and the systemic blood pressure builds up. The ductus ordinarily closes against and in spite of this stream, because it is its nature to do that particular thing if the force of the stream is not too great.

SUMMARY

Intraventricular pressures were taken from the right and left ventricles of immature and mature dog and rabbit fetuses immediately upon delivery, when the umbilical cord was clamped, when the fetus first breathed, and during the first two days of independent life.

1. Right and left ventricular pressures were similar before breathing begins. They were less in the immature fetuses than in the mature.

2. Clamping the cord produced little or no change in right or left ventricular pressure.

3. Inspiration lowered right ventricular pressure more than left.

4. Pressure relationships were such that one must postulate an early functional closure of the foramen ovale and ductus arteriosus in these species.

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CATALEPTIC SYMPTOMS FOLLOWING BILATERAL CORTICAL LESIONS IN CATS

RALPH W. BARRIS[1]

From the Department of Anatomy, School of Medicine, George Washington University

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A peculiar syndrome, in many ways suggestive of catalepsy, has been observed in cats following a one-stage operation in which the rostral regions of the cerebral cortex were bilaterally removed. Catalepsy has been defined as a state in which there are sudden suspensions of voluntary motion, lowered sensibilities, and a high degree of plasticity of the somatic musculature. By virtue of such plasticity, the limbs of an individual will retain a passively-induced posture for long periods of time.

The observations on animals herein described were made on both acute and chronic preparations. Six animals were observed over periods ranging from one to two weeks; four animals over periods of two to four weeks; and three animals over periods of ten to twelve weeks.

METHODS. All operations were carried out aseptically under Nembutal anesthesia (pentobarbital sodium, 0.2 grain per pound). As a rule, the bony roof of the frontal sinus was first removed, the naso-frontal ducts securely blocked with a bony plug, and the sinuses dried with gauze sponges moistened with Merthiolate (alcoholic solution, 1:1000). The bony covering of the rostral part of the cranial vault was then removed, the posterior wall of the frontal sinus rongeured away, and the dura incised so as to expose the surface of the rostral one-fourth of the neo-cortex. Bilateral surgical removal of the neo-cortex lying rostral of a point two to three millimeters behind the medial tip of the ansate sulcus was achieved in the following manner. With the aid of a sharply-pointed scalpel, an initial incision was directed laterally as far as the caudal part of the orbital sulcus. This incision was from three-sixteenths to one-fourth of an inch in depth. A narrow, thin-bladed spatula was then used to elevate the cortex lying anteriorly. This cortical zone was then carefully undercut, the incision being carried forward and somewhat downward. The isolated slab of cortical tissue was then elevated and removed. The complete removal of the gyrus proreus was not always achieved, but as a rule its upper half, at least, was destroyed (figs. 9 and 11). Good hemostasis was secured, following which the corresponding cortical region on the side opposite was simi-

[1] Aided by a grant of the National Research Council.

larly removed. The temporalis muscles of the two sides were drawn up along the two walls of the skull and fastened by silk sutures extending across the midline of the dorsal surface of the intact skull. Following closure of the wound with metal clips, a subcutaneous injection of 100 cc. of sterile saline solution was administered to each animal. It was then placed in a warm compartment, free of draughts.

Observations were recorded approximately twenty-four hours later and repeated almost daily for the first two weeks. The status of the animals became fairly constant after this interval of time, but additional observations were recorded from time to time thereafter.

Observations. The outstanding abnormality as observed in these animals was the presence of plasticity in the somatic musculature, by virtue of which an animal could be made to assume abnormal postures for long periods of time. When these animals were placed upon their backs in a wooden trough-like arrangement, plasticity of the limb musculature and of the muscles of the back could be especially well demonstrated. Such plasticity was always most pronounced during the first week or two following operation. A definite deficiency in the ability to initiate and maintain movements of the extremities such as, for example, might be employed in the act of walking, was apparent throughout the survival periods of all animals. These animals always appeared rather negative in their general emotional reactions. If, for example, they were confronted with a barking and snapping dog, they for the most part seemed quite unconcerned and never reacted in the fashion typical of a normal animal under the same circumstances. Their reactions to ordinarily painful stimuli, such as pinching the tail, were likewise subnormal. A very definite deficiency in certain of the righting reactions was always observable and could be easily detected in all of the animals by applying appropriate methods of testing. If, for example, they were dropped from their backs into an outstretched canvas, they either did not right themselves at all, or the reaction was delayed and incomplete.

General behavior. The general impression of stolidity characterized the appearance of these animals. Their reactions to environmental situations were always rather negative. They usually stood about the room in some absurd posture staring rather vacuously at their surroundings. Their responses to painful stimulation were decidedly subnormal. Many animals, especially during the first week or two of their survival, failed to show the slightest reaction to pinching of the tail. Sometimes they would utter but a feeble cry. In the later part of their survival periods, severe pinching of the tail often produced movements of a weakly defensive nature, such as a turning of the body partially around in a semi-crouching position, together with a rather ineffectual effort to use the forelimbs defensively. In two animals, a very moderate erection of the hair with

moderate pupillary dilatation was observed after severe pinching of the tail. This was several weeks after operation, and these two animals under these circumstances could be made to "hiss" rather weakly. Repeated observations of all animals gave the impression, however, that the expression of emotion in terms of activity of the sympathico-adrenal mechanism was subnormal.

These animals did not lick their coats or otherwise care for themselves. One animal, however, was observed after a survival period of several weeks to occasionally lick the hair at the base of the neck. Usually, this act was inefficiently performed, the licking motions of the tongue being carried out without actually touching the hair at the base of the neck.

For a week or more following operation, feeding by stomach tube was usually necessary. Many animals required artificial feeding throughout their survival periods. One animal, still living, has been fed by tube for ninety-three days. When an animal could be induced to eat voluntarily, it was usually necessary to place the food receptacle directly in front of it and often its snout had to be dipped into the food dish. Food was thrown into the mouth by a peculiar "licking" motion of the tongue. Frequently, they would suddenly stop eating, walk through their food dishes, come to a halt, look stupidly about, and need to be returned by the observer to the food receptacles. Their appetites seemed exceptionally good, however, and when proper circumstances were provided, they ate with evident relish.

Plasticity. During the first one or two weeks, the animals demonstrated a remarkable plasticity of the limb and back musculature. This could be best demonstrated if the animal was placed in a supine position with the back of the head resting against the bottom of a wooden trough. Although all animals always showed a definite tendency to assume a "curled up" position when laid on their backs, they would lie utterly motionless following application of gentle pressure requisite to place them fully on their backs in the trough.

Here they would lie, breathing slowly and regularly, eyes usually open and following the observer, for long periods of time. As a rule, the limbs would be in the extended position (fig. 4). There was good resistance to passive flexion of the limbs, and the limbs retained the flexed position. If, later, they were passively extended, there was good resistance to passive extension, the limbs remaining extended throughout the period of quietude of the animal.

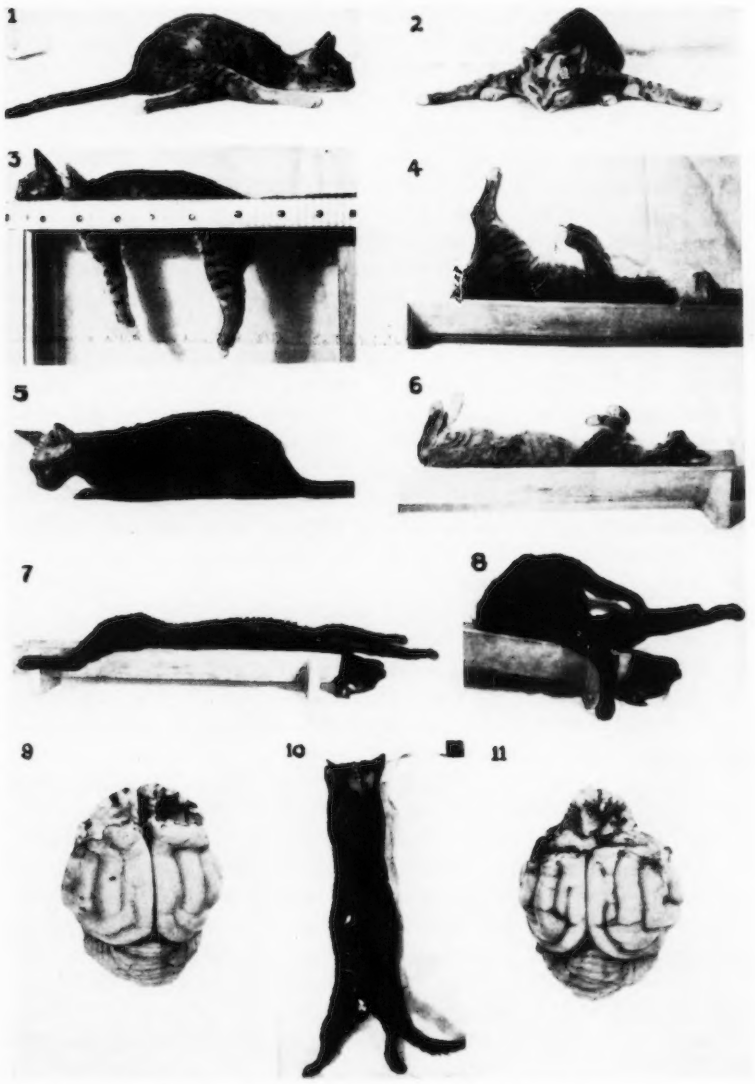
The hind limbs could be carried over the fore part of the body by passive elevation of the pelvic region, and the animals would retain a very bizarre postural attitude (fig. 8). Various types of other peculiar attitudes could also be passively imposed (fig. 7). Such attitudes would sometimes be maintained for hours, certainly in what would be a very uncomfortable, if not painful posture, for a normal animal.

After the first one to two weeks, it became less easy to induce an animal to lie quietly in the supine position. But if after placing an animal upon its back, the head was gently restrained, it would lie quietly making no attempt whatever to escape from the trough. In the later survival periods, the limbs would as a rule be semi-extended when the animal was placed upon its back. There was fair resistance to passive flexion of the limbs and they usually maintained a semi-flexed position. Likewise, there was a fair resistance to passive extension of the limbs and good maintenance of the semi-extended positions.

In thefirst week of the survival periods, most animals could be "moulded" into odd postures when in the upright position on the floor. For instance, if the observer widely abducted all limbs of an animal, when either crouching or standing, the limbs would remain in this very abnormal position for long periods of time. If, instead, the forelimbs were passively abducted and the hindlimbs passively extended forward beneath the animal, such a posture might also be maintained for considerable periods (fig. 2). Various other modifications of posture could frequently be imposed at will on the crouching or standing animal (figs. 1, 5 and 10).

Motor initiative. Observations made upon these animals suggest that there may be a deficiency or an impairment of those volitional elements having to do with the initiation and maintenance of such normal acts as walking and running. It has been mentioned that these animals may stand for hours at a time in one posture, whether passively imposed or voluntarily assumed. Under these circumstances, animals have been repeatedly observed to rather abruptly shift their centers of gravity forward as if in preparation for walking, only to suddenly relax, apparently lacking the volitional element requisite to walking forward. After several abortive attempts, these animals succeed in walking forward, even though their gait is not perfectly normal. Once engaged in walking, these animals finally come to a halt with rather striking suddenness, the limbs remaining in abnormal positions. To the observer it appears as if there was a sudden inhibition of some factor necessary to continuous performance. When, again, these animals have been placed in a supine position, motor performance has been utterly repressed for long periods. But, suddenly and without warning, these animals may extricate themselves from this position, turning over on their sides, assume an upright position, and walk vigorously for several steps. Then, they may halt suddenly, their limbs remaining fixed in positions suggestive of the walking posture.

To assume that volition plays a part in the normal acts of walking and other motor performances, is not to suggest that the act of walking or motor performance is necessarily dependent upon a cortical mechanism in the normal animal. For it has been shown and amply verified by many investigators that the act of walking is largely carried out by reflex mecha-



Figs. 1 and 2. Passively imposed posture of cat, three days after operation.

Figs. 4 and 6. Showing position of cat in trough following passive extension and passive flexion, respectively, of extremities.

Fig. 3. Position in hammock, showing extensor position of limbs.

Figs. 5, 7, 8 and 10. Showing various passively induced postures.

Figs. 9 and 11. Brains of two cats, showing extent of cortical removal.

nisms operating at subcortical levels. That the cortex may exert a modifying influence on such reflex behavior seems evident in the light of the observations herein reported.

Hypertonus without plasticity. All animals when supported in a canvas hammock exhibited a marked extension of the limbs and as a rule made no attempt whatever to escape (fig. 3). In this device, an animal is so supported that the ventral body surface rests upon a stretch of canvas, the limbs hanging downward through properly-arranged openings. A normal cat, when placed in such a hammock, usually shows a flexion of all limbs and will struggle to free itself. Regardless of the length of the survival period, an extensor rigidity of the limbs was observed in the animals herein considered when they were placed in the hammock. When upward pressure was applied to such limbs, the animal was usually raised out of the hammock. If, however, one hand was placed over the shoulders or dorsal pelvic region, the fore or hind limbs might by upward pressure be passively flexed against a very good resistance. Upon releasing the upward pressure, however, there was always an immediate re-extension of the limbs.

This failure of all limbs to maintain a passively-flexed position under these circumstances argues against the presence of plasticity in the flexor muscles of the limbs. This phenomenon is certainly in marked contrast to the plastic character of the limbs when the animal is in the supine position. The vigor with which a passively-flexed limb is re-extended when the animal is resting in the hammock certainly argues against the existence of plasticity in the extensor muscles. It is entirely possible that the immediate resumption of an extended position of the limbs following passive flexion is dependent upon some reflex activity initiated by sensory stimulation of the body wall.

Discussion. Ranson and Ingram (1932) and Ingram, Barris and Ranson (1936) have observed cataleptic symptoms in cats following restricted lesions in the retro-mammillary region. Ranson and Ingram (1932) pointed out that their animals "exhibited an increased muscle tonus of an extremely plastic type by virtue of which they would assume and maintain any posture in which it pleased the experimenter to mould them. The plasticity could be especially well demonstrated when the cats were resting on their backs in a shallow trough. If the limbs were passively flexed, they remained flexed. If passively extended, they remained passively extended indefinitely, and considerable resistance was required to return them again to the flexed position. In spite of this marked resistance to flexion, which was as great as any seen in the best decerebrate preparations, the limbs, when they had once been passively flexed, exhibited no tendency to extend themselves. These cats . . . were much more plastic than any decerebrate preparations which we have examined."

When it is remembered that the above description refers to animals with

injuries to the retro-mammillary region, it is evident that there is a similarity between the behavior of such animals and those in which the rostral portions of the cerebral neo-cortex have been removed. However, in the animals observed by the above investigators, there were many evidences of somnolence, a symptom which was lacking in the animals under consideration in the present report.

Fulton, Jacobsen, and Kennard (1932) reported observations on two monkeys from which had been removed in one stage all cortical tissue anterior to the excitable motor area; that is, all cortical tissue anterior to Brodmann's area 4. These investigators reported that within one or two days after operation both animals began to show perseveration, slowness of movement, and striking retardation in the initiation of movement. They pointed out that these animals also showed forced groping and grasping, which they believed to be a sign of a much more profound underlying disturbance, involving difficulty in the initiation of voluntary movements, perseveration, and the diminished ability to execute highly skilled movements. In reporting the post-operative behavior of one of these monkeys, it was stated that during the first few days it would stand for hours at a time with head slightly depressed, gazing vacantly into space, and that all purposeful movements were noticeably slow in starting during the first three weeks.

From a comparison of the above description of monkeys deprived of all cortical tissue anterior to Brodmann's area 4 with cats deprived of almost all of the more rostral regions of the neo-cortex, it appears that there are a number of similarities. It should be emphasized that the cataleptic element of plasticity of the musculature is best demonstrated in cats following a *one-stage* operation for removal of the rostral portion of the neo-cortex. Although the differences which exist with reference to plasticity of the musculature of the extremities between uni-laterally and bi-laterally operated animals will be the subject of a later report, it might be well to point out that while there is some evidence of a low-grade plasticity of the contra-lateral extremities following uni-lateral removal of the rostral portion of the neo-cortex, it is not nearly as marked nor as lasting as after bi-lateral cortical destruction.

One might suspect that many of the symptoms of cataleptic behavior which have been described herein for cats following bi-lateral one-stage removal of the rostral portion of the neo-cortex were due to hemorrhagic involvement of brain-stem structures. While such an opinion is plausible, the most careful examination for evidences of hemorrhage at the time of autopsy of the greater number of this series of animals failed to reveal any evidences of hemorrhagic involvement of the brain-stem or of blood within the ventricles. Furthermore, all thirteen of the animals in the present experiments showed the same qualitative symptoms, there being only

slight quantitative differences. The nature of the symptoms was a constant finding in all animals regardless of their period of survival, even though quantitatively there was some diminution in the second, third, and fourth weeks. A further study of animals lacking more restricted areas of the cerebral neo-cortex is now in progress, in the hope that such observations will serve to delineate more precisely the cortical areas concerned in the very peculiar character of the symptoms of animals described in the present report.

SUMMARY

Bilateral one-stage removal of the rostral portions of the neo-cortex of cats results in a group of symptoms closely resembling catalepsy. A high degree of plasticity of the musculature of the extremities and back characterizes this condition. By virtue of such plasticity, these animals may be moulded into very abnormal postures and attitudes which are retained for considerable periods of time. The ability of these animals to maintain or initiate movement of the extremities seems definitely subnormal.

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